Efficacy of systemically administered polymyxins in mouse burn wound infection caused by multidrug-resistant Gram-negative pathogens: A proof-of-concept study

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Running title: Efficacy of polymyxins for burn wound infections 

Key Words: Polymyxins, thermal injury, multidrug-resistant, Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae.
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 achieved a >2-log₁₀ reduction in the bacterial load for *P. aeruginosa* and *A. baumannii* infections, while wound infections by *K. pneumoniae* were less responsive (<1-log₁₀ reduction).

This study highlights the potential therapeutic benefits of parenteral polymyxins for treating burn wound infections.
Thermal injury is a major global public health problem that is associated with high mortality and morbidity rates (1-4). Approximately 50% of deaths owing to thermal injury is 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 infections 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 have undergone clinical resurgence as a last resort (10, 11). Two polymyxins are used in the clinic: polymyxin B and colistin with similar *in vitro* pharmacodynamic (PD) properties (12). Although polymyxins are being increasingly used for treating systemic infections caused by these MDR pathogens, very few studies have investigated the efficacy of systemically administered polymyxins for treating burn wound infections. The present study aimed to examine the antimicrobial efficacy of systemically administered polymyxins against burn wound infections caused by MDR *P. aeruginosa*, *A. baumannii* and *K. pneumoniae*.

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, 8-10 weeks; weight 25-30 g) were obtained from Monash Animal Services (Clayton, Victoria, Australia). A neutropenic mouse burn wound infection model was established as previously described (13,
with *P. aeruginosa* (PAO1 and 19056muc; colistin and polymyxin B MIC = 1 and 0.5 mg/L, respectively), *A. baumannii* (ATCC 19606 and 248-01-C.248; colistin and polymyxin B MIC = 1 mg/L for both isolates), and *K. pneumoniae* (ATCC BAA 2146 and KP1; colistin and polymyxin B MIC = 0.5 mg/L for both isolates). MICs were determined by broth microdilution (15). Mice were rendered neutropenic by subcutaneous injection of two doses of cyclophosphamide on Day -4 and -1 (16). While under anaesthesia with gaseous isoflurane, a third-degree thermal injury was induced by pressing a heated block (92 - 95°C, diameter = 1.4 cm) to the shaved back of a neutropenic mouse for approximately 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; ≥15000 IU/mg; Sigma-Aldrich, MO, USA) and polymyxin B sulfate (Lot BCBF8382V; ≥6000 units/mg; Sigma-Aldrich, MO, USA) were subcutaneously administered at 2 h post inoculation. Colistin or polymyxin B 30 mg/kg thrice daily (8 hourly, 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 start of the treatment. The entire burn wound tissue was aseptically collected and individually processed as previously described (16). The bacterial load of each wound tissue was expressed as log_{10} CFU per wound tissue. The lower limit of the colony count was 170 CFU per wound (equivalent to one colony per plate). Statistical analysis was performed using Student’s t-test (Graph Pad Prism Version 7.00, San Diego, CA, U.S.A.).
The dosage regimens of polymyxins used were chosen based on their plasma PK in critically ill patients and animal scaling (17-19). With the currently recommended dosage regimens, parenteral administration of colistin (as CMS) and polymyxin B results in an average steady-state unbound concentrations \( (fC_{ss,\text{avg}}) \) of 1 to 3 mg/L (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 \( (fAUC_{0-24}) \) of approximately 20-33 mg·h/L which is equivalent to \( fC_{ss,\text{avg}} \) of ~0.8-1.5 mg/L for both polymyxins (16, 23). A limitation of the present study is that single-dose PK of polymyxins was not conducted 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 intravenous 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 (diameter 1.4 cm) was only approximately 1.92% of the total body surface area in mice (24, 27). Therefore, it is unlikely that the PK of both polymyxins was substantially altered in our burn wound mouse infection model. Nevertheless, our current study revealed that subcutaneously administered polymyxins (30 mg/kg thrice daily) were able to effectively decrease the bacterial load by \( \geq 2\text{-log}_{10} \) in thermally injured mice infected with \( P. \text{aeruginosa} \) or \( A. \text{baumannii} \) (Figure 1). This is consistent with the previous observations in thermally injured patients, in which parenteral administered CMS (5 mg/kg/day; maximum 160 mg every 6 h)
displayed positive clinical outcomes and prevented sepsis in thermally injured patients by reducing the endotoxin levels (28, 29).

The antibacterial efficacy of colistin versus polymyxin B for treating burn wound infections caused by Gram-negative pathogens was shown in Figure 1. There was no significant difference in the antibacterial efficacy between colistin and polymyxin B for all isolates (p > 0.05). These results are similar to our previous in vivo study, in which equimolar daily doses of each polymyxin resulted in similar antibacterial efficacy for the K. pneumoniae ATCC BAA 2146 and FADDI-KP032 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 an inactive prodrug, CMS, while only formed colistin is the antibacterial entity (10, 32). A clinical PK study in health subjects showed that the CMS-to-colistin conversion was relatively slow and low following intravenous administration (33). In contrast, polymyxin B is administered in its pharmacologically active form (11, 34). This rapid attainment of target polymyxin B concentration is linked to the superior bacterial killing in a one-compartment in vitro model (35). Future clinical studies are needed to examine which of the two clinically available polymyxins is superior in treating burn wound infections.
fAUC/MIC was previously identified as the most predictive PK/PD index that described the antibacterial efficacy of polymyxins against *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* in a mouse thigh infection model (16, 23). Both polymyxin B and colistin share similar PK/PD targets against the same isolate because of the similar in vivo PK/PD properties and molecular weights (16, 23). A good correlation between the previously established fAUC/MIC targets for thigh infections (16) and the PD outcome for treating burn wound infections has been demonstrated in the present study. Assuming that the small-area thermal injury had no major effect on the PK of polymyxins in mice, 90 mg/kg polymyxin B and colistin would achieve a polymyxin fAUC/MIC value of 66-40 for strains with an MIC of 0.5 mg/L or 33-20 for strains with an MIC of 1 mg/L (Table 1), which were much higher than the fAUC/MIC target of 7.4-17.6 against *P. aeruginosa* and *A. baumannii* for a 2-log₁₀ reduction in a neutropenic mouse thigh infection model (16, 23). In agreement with these PK/PD targets, a >2-log₁₀ reduction was achieved in the present study for the burn wound infection caused by *P. aeruginosa* and *A. baumannii* (Figure 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/daily), a 2-log₁₀ reduction was not achieved against *K. pneumoniae* (23). Similarly, our present study also demonstrated that a 2-log₁₀ reduction was not achieved against *K. pneumoniae* in thermally injured mice with 30 mg/kg/8h colistin or polymyxin B (Figure 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...
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 have demonstrated that previously established PK/PD targets against mouse thigh infection are useful to guide the selection of the optimal dosage regimens for treating burn wound infections. Well-designed clinical studies are warranted to investigate the likely impacts of thermal injury on the PK of polymyxins and their potential therapeutic efficacy in burn patients.

**Acknowledgements**

J.L. is an Australian National Health and Medical Research Council (NHMRC) Senior Research Fellow. T.V. is an Australian NHMRC Industry Career Development Level 2 Research Fellow. This study was conducted as part of our routine work. J.L. and T.V. are supported by a research grant from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (R01 AI132154). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health.
Transparency declarations

None to declare.
Colistin and PMB dosage regimen involved 30 mg/kg thrice daily to achieve $f_{AUC} = \approx 33 \text{ mg}\cdot\text{h}/\text{L}$ (16) and $\approx 20 \text{ mg}\cdot\text{h}/\text{L}$ (23), respectively.

$f_{AUC}/\text{MIC}$ is the area under the unbound polymyxin concentration-time curve over 24 h divided by the MIC.
Values are achieved fAUC/MIC following treatment with 30 mg/kg thrice daily, which were calculated based on the assumption that mild thermal injury had no significant effect on the PK of polymyxins in mice (25).
Figure 1. Efficacy of subcutaneously administered colistin and polymyxin B (PMB) therapy (30 mg/kg thrice daily) at 24 h after start of treatment against burn wound infections caused by (A) *P. aeruginosa* PAO1, (B) *P. aeruginosa* 19056muc, (C) *A. baumannii* ATCC 19606, (D) *A. baumannii* 248-01-C.248, (E) *K. pneumoniae* ATCC BAA 2146, and (F) *K. pneumoniae* KP1. Thermally injured mice treated with sterile saline were included as growth controls at 0 h and 24 h. The broken line represents the limit of detection (2.21 Log10 CFU/wound tissue). Data = mean ± standard deviation (n = 3).
References


