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Polymyxin B and Fosfomycin Thwart KPC-producing *Klebsiella pneumoniae* in the Hollow Fibre Infection Model

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Abstract

Polymyxin B and fosfomycin are two ‘old’ antibiotics that consistently maintain activity against *Klebsiella pneumoniae* carbapenemase (KPC)-producing organisms based on *in vitro* susceptibility testing. However, each antibiotic’s use in monotherapy has been associated with high rates of treatment failure. Therefore, our objective was to investigate the combinatorial pharmacodynamics of polymyxin B and fosfomycin against KPC-producing *K. pneumoniae*. Polymyxin B front-loading (3.33 mg/kg for 1 dose followed by 1.43 mg/kg q12h starting 12h later) and burst (5.53 mg/kg for 1 dose followed by no subsequent doses) simulated dosing regimens were explored in combination with fosfomycin (4g q8h) against KPC-2-producing *K. pneumoniae* ST258 in a hollow fibre infection model over 120h. Population analysis profiles were used to track the temporal polymyxin B and fosfomycin resistance profiles. Against isolate KPC-Kp 9A (polymyxin B MIC: 0.5mg/L, fosfomycin MIC: 8mg/L), monotherapies resulted in >3 log₁₀CFU/mL killing within 3h, but regrowth and proliferation of resistant subpopulations within 48h. Polymyxin B combinations with fosfomycin demonstrated rapid bacterial killing (>6 log₁₀CFU/mL reductions) while preventing propagation of polymyxin and fosfomycin resistance. Against isolate KPC-Kp 24A with a higher fosfomycin MIC (polymyxin B MIC: 0.5mg/L, fosfomycin MIC: 32g/L), a polymyxin B burst and fosfomycin combination caused a >6 log₁₀CFU/mL reduction within 1 hour, although bacterial regrowth occurred with the amplification of fosfomycin-resistant subpopulations. Polymyxin B in combination with

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fosfomycin may provide a practicable treatment strategy against KPC-producing *K. pneumoniae* and warrants further investigation.

Keywords

polymyxin B; fosfomycin; PK/PD; carbapenemase; KPC-producing *K. pneumoniae*

1. Introduction

Klebsiella pneumoniae carbapenemase-producing *K. pneumoniae* (KPC-Kp) have emerged as an important cause of infection worldwide. The sequence type 258 (ST258) lineage of *K. pneumoniae* has become the dominant strain among KPC-Kp internationally, causing unacceptable mortality with rates as high as 50% (1). This excessive mortality persists despite a low inherent KPC-Kp virulence, which suggests that optimization of antimicrobial therapy may be beneficial. Given the dearth of novel agents active against carbapenemase-producing organisms, there is a dire need to optimize ‘old’ antibiotics that have retained antibacterial activity.

The polymyxins (polymyxin B and colistin), an ‘old’ class of antibiotics, serve as a last line of defence to combat multidrug-resistant Gram-negative bacteria including KPC-Kp. The polymyxins destabilize the outer membrane through electrostatic and hydrophobic interactions with the lipopolysaccharide layer leading to increased permeability and cell death. However, dose-limiting nephrotoxicity is common and may be related to prolonged, high-dose exposure to polymyxin antibiotics (2). Fosfomycin is another ‘old’ antibiotic that has a unique mechanism of bactericidal activity involving termination of cell wall biosynthesis. Fosfomycin has also been shown to reduce the incidence of nephrotoxicity of co-administered antibiotics such as vancomycin and aminoglycosides in a dose-dependent fashion (3). Despite potent *in vitro* activity and high susceptibility rates to the polymyxins and fosfomycin, there remains a discordance between experimental data and clinical outcomes for patients with KPC-Kp infections who receive monotherapy with either agent (4). The poor outcomes for polymyxins and fosfomycin when used alone are likely in part due to their tendency to cause proliferation of antibiotic resistance. Combination treatment has previously been shown to improve microbiological and clinical outcomes compared to monotherapy, although consensus therapies have yet to be completely defined (5, 6). In the present study, the combinatorial pharmacodynamics of a pair of resurgent antibiotics, polymyxin B and fosfomycin, was explored against clinical KPC-Kp bloodstream isolates.

2. Methods

KPC-2 producing *K. pneumoniae* ST258 bloodstream isolates (KPC-Kp 9A and KPC-Kp 24A) were used. Fresh antibiotic stock solutions of polymyxin B (Sigma Aldrich, St. Louis, MO; Lot Number WXBB4470V) and fosfomycin (Sigma Aldrich, St. Louis, MO; Lot Number BCBN2137V) were made prior to each experiment. Glucose-6-phosphate (G6P; Sigma Aldrich, St. Louis, MO; Lot Number 031M7020) 25 mg/L was added to all growth media. Susceptibility testing was performed per the European Committee on Antimicrobial Susceptibility Testing (EUCAST); MICs for polymyxin B were determined using broth

microdilution in duplicate while fosfomycin MIC values were obtained in duplicate by agar dilution on Mueller-Hinton agar supplemented with G6P (7). *K. pneumoniae* resistance to polymyxin B and fosfomycin was defined according to the EUCAST cutoff for Enterobacteriaceae as having MICs greater than 2 and 32 mg/L, respectively. KPC-Kp 9A and KPC-Kp 24A were both susceptible to polymyxin B (MICs: 0.5 mg/L) and fosfomycin (MICs: 8 and 32 mg/L, respectively).

Hollow fibre infection model (HFIM) experiments were performed as previously described (8) to evaluate the pharmacodynamics of polymyxin B dosing regimens alone or combined with fosfomycin. Specifically, an overnight bacterial suspension was adjusted in Mueller-Hinton broth supplemented with calcium (25 mg/L) and magnesium (12.5 mg/L) to obtain a $\sim 10^6$ CFU/mL initial inoculum for placement into the extracapillary space of the cellulosic cartridge (C3008; FiberCell Systems Inc., Frederick, MD). Bacterial samples were collected serially over 120h for viable total population counting. Population analysis profiles (PAPs) were also performed throughout the HFIM (0, 24, 48, 72, 96 and 120h) to assess the suppression or amplification of polymyxin B and fosfomycin heteroresistant subpopulations by plating isolates on MHA imbued with polymyxin B (1, 4, and 10 mg/L polymyxin B for experimental arms containing polymyxin B) or fosfomycin + 25mg/L G6P (8, 32, and 128 mg/L fosfomycin for experimental arms containing fosfomycin). Polymyxin B was supplemented to maintain a pharmacokinetic profile consistent with an 8h half-life (9). The following regimens were mimicked alone or in combination in the HFIM to simulate the disposition of polymyxin B ($t_{1/2}=8$ h) (8, 10) and intravenous fosfomycin ($t_{1/2}=2$ h) (11, 12) in critically ill patients:

- I. Polymyxin B Front-Loading:** 3.33 mg/kg for 1 dose followed by 1.43 mg/kg q12h starting 12h later, with an $fC_{max}=3.61$ mg/L and $fAUC_{0-24}=48.5$ mg·h/L across the first day and $fC_{max}=2.41$ mg/L and $fAUC_{24}=35.9$ mg·h/L at steady state.
- II. Polymyxin B Burst:** 5.53 mg/kg for 1 dose followed by no subsequent doses with an $fC_{max}=6.0$ mg/L and $fAUC_{0-24}=60.4$ mg·h/L.
- III. Fosfomycin:** 4g q8h with an $fC_{max}=150$ mg/L and $fAUC_{24}=1330$ mg·h/L.

Samples to validate polymyxin B and fosfomycin concentrations in the HFIM were collected over 48h in an experiment conducted before bacteria were added. Pharmacokinetic samples were then immediately stored at -80°C until analysis. Polymyxin B concentrations were determined by a liquid chromatography single quadrupole mass spectrometry (LC-MS) method as previously described (13). Fosfomycin concentrations were determined by microbiological assay using *Staphylococcus aureus* (ATCC 29213) as an indicator organism, diluted to 10^6 CFU/mL in Difco #5 agar with 25mg/L G6P. Using 35 mL of agar, fosfomycin concentrations ranging from 5 to 200 mg/L were used to generate a logarithmic standard curve ($R^2>0.99$).

3. Results

The pharmacodynamic activities of polymyxin B and fosfomycin as monotherapies or in combinations against KPC-Kp 9A in the HFIM are displayed in Figure 1A. Against KPC-

Kp 9A, exposure to either a polymyxin B front-loading or fosfomycin regimen caused maximal bacterial reductions at 3h of 4.44 and 3.40 log₁₀CFU/mL, respectively, followed by regrowth to near growth control levels by 24h. However, administration of polymyxin B as a burst resulted in greater killing and delayed regrowth compared to either the polymyxin B front-loading or fosfomycin monotherapies. The burst alone produced undetectable total population counts at 4h (6.05 log₁₀CFU/mL reduction) and regrowth to growth control levels was delayed until 48h. Polymyxin B-fosfomycin combinations against KPC-Kp 9A displayed rapid bactericidal activity of >6 log₁₀CFU/mL reductions within 2h. The combination was also markedly synergistic between 24 and 120h where total population counts remained near the limit of quantification. PAPs revealed rapid proliferation of polymyxin B and fosfomycin-resistant subpopulations following exposure to monotherapies (Figure 1B). Conversely, combination regimens successfully suppressed both polymyxin B and fosfomycin-resistant subpopulations.

In an attempt to corroborate the remarkable bactericidal killing observed for the polymyxin B burst and fosfomycin combination against KPC-Kp 9A, this combination was assessed against an isolate with a higher fosfomycin MIC, KPC-Kp 24A (Figure 2A). Within 1h of administering the polymyxin B burst together with fosfomycin, KPC-Kp 24A total population counts became undetectable (6.12 log₁₀CFU/mL reduction) and remained below the limit of quantification for 50h. Regrowth of KPC-Kp 24A to near growth control levels was observed for the polymyxin B burst and fosfomycin combination, but not until ~78h. In contrast to the suppression of resistance seen with KPC-Kp 9A, PAPs performed during combination therapy revealed that there were fosfomycin-resistant subpopulations of KPC-Kp 24A that proliferated concurrently with regrowth (Figure 2B). However, the polymyxin B burst and fosfomycin combination was able to suppress bacterial growth on polymyxin B containing agar to levels below that of the growth control.

4. Discussion

Therapeutic treatment strategies to combat carbapenemase-producing Gram-negatives such as KPC-Kp are extremely limited. The polymyxins and fosfomycin have both been thrust back to the forefront of clinicians' armamentarium because of its persistently high rates of activity against KPC-Kp (14, 15). Although polymyxin B and fosfomycin are infrequently utilized together clinically, the excellent susceptibility profiles of both 'old' antibiotics against KPC-Kp makes these antibiotics a potentially promising pair. The present study is the first to examine polymyxin B and fosfomycin combinatorial pharmacodynamics in the HFIM against KPC-Kp.

Here, we determined that the combination of a high single-dose polymyxin B burst together with fosfomycin demonstrated extensive bacterial-killing activity against KPC-Kp. Unlike colistin methanesulfonate (CMS), which is an inactive pro-drug that requires *in vivo* conversion to the active form, administering polymyxin B can rapidly generate higher plasma concentrations to maximize bacterial killing. The data from the present study suggests that the use of a polymyxin B one-time burst dose with fosfomycin may result in comparable bacterial killing to the combination with polymyxin B administered in a more traditional fashion (front-loading) while concurrently decreasing overall drug exposure.

Furthermore, the polymyxin B burst containing combination displayed repression of polymyxin resistance compared to monotherapies and growth controls suggesting the benefit of fosfomycin co-administration is multifactorial. This repression of polymyxin resistance by fosfomycin is similar to the effect by rifampin that we recently described for KPC-Kp (16). Abdelraouf *et al.* showed that once daily dosing of polymyxin B leads to lower kidney tissue drug concentrations and was less nephrotoxic than 6 hourly dosing in a rat model (17). Therefore, by reducing the duration and overall exposure to polymyxin B (the $fAUC_{24-48h}$ was 7.8 mg.h/L and undetectable on subsequent days), the high intensity, single dose burst regimen holds promise in its ability to reduce overall drug exposure and nephrotoxicity (2).

Interestingly we noted variation in killing detected between the two KPC-Kp isolates studied, both being susceptible to polymyxin B and fosfomycin. This may be due to the higher proportion of KPC-Kp 24A fosfomycin-resistant subpopulations observed at baseline and a correspondingly higher fosfomycin MIC. The amplification of fosfomycin-resistant subpopulations has previously been proposed in *Pseudomonas aeruginosa* as a mechanism for fosfomycin failure (18). To overcome fosfomycin heteroresistance, higher fosfomycin doses beyond the 12g/day tested here may be warranted to achieve longer time above the resistance inhibitory concentration; a marker which has been shown to predict adequate fosfomycin pharmacodynamic activity *in vitro* (19). Higher fosfomycin concentrations could also prevent the emergence of fosfomycin resistance where an inverse relationship between concentration and frequency of resistance has previously been reported for *K. pneumoniae* (20).

4. Conclusions

In conclusion, we have shown that human-simulated concentrations of polymyxin B and fosfomycin in combination are capable of causing more sustained bactericidal killing of KPC-Kp compared to either drug as monotherapy. Administering fosfomycin in combination with polymyxin B not only enhanced killing but also repressed polymyxin resistance. To our knowledge, the present study represents the first analysis of polymyxin B and fosfomycin combinations against KPC-Kp in a dynamic *in vitro* model. Our findings support that the pairing of two ‘old’ antibiotics, polymyxin B and fosfomycin, may provide a viable treatment strategy in the war against KPC-Kp susceptible to polymyxin and fosfomycin. Confirmation in additional KPC-Kp isolates and in animal models must validate the present findings prior to evaluation in the clinic.

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Figure 1.

Tracking KPC-Kp 9A resistance and viability in the 5-day HFIM following exposure to polymyxin B front-loading (3.33 mg/kg for 1 dose followed by 1.43 mg/kg q12h starting 12h later), polymyxin B burst (5.53 mg/kg x 1 dose followed by no subsequent doses), fosfomycin (4g q8h), or polymyxin B-fosfomycin combinations against a $\sim 10^6$ CFU/mL inoculum. **(A)** Total populations represent viable colony counts on MHA following exposure to each corresponding regimen, whereas **(B)** resistant subpopulations represent growth on MHA plates imbued with polymyxin B (blue) or fosfomycin (red) throughout the HFIM experiment (Note: panels B1-B6 also display their respective total population lines from panel A).

A) KPC-Kp 24A Total Populations

B) KPC-Kp 24A Resistant Subpopulations

Figure 2.

Tracking KPC-Kp 24A resistance and viability in the 5-day HFIM following exposure to polymyxin B administered as a burst (5.53 mg/kg x 1 dose followed by no subsequent doses) in combination with fosfomycin (4g q8h) against a $\sim 10^6$ CFU/mL inoculum. (A) Total populations represent viable colony counts on MHA following exposure to the polymyxin B-fosfomycin combination (purple) versus growth control (black), whereas (B) resistant subpopulations represent growth on MHA plates imbued with polymyxin B (blue) or fosfomycin (red) throughout the HFIM experiment (Note: panels B1 and B2 also display their respective total population lines from panel A).