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Synergistic combinations of polymyxins

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ABSTRACT

The proliferation of extensively drug-resistant Gram-negative pathogens has necessitated the therapeutic use of colistin and polymyxin B. However, treatment failures with polymyxin monotherapies and the emergence of polymyxin resistance have catalysed the search for polymyxin combinations that synergistically kill polymyxin-susceptible and -resistant organisms. This mini-review examines recent (2011–2016) in vitro and in vivo studies that have attempted to identify synergistic polymyxin combinations against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii*. Clinical evidence for the use of combination regimens is also discussed.

1. Introduction

After decades of antimicrobial exposure, multidrug-resistant (MDR) pathogens are now emerging with resistance to three or more antibiotic classes [1,2]. Even more troubling are extensively drug-resistant (XDR) Gram-negative pathogens that are non-susceptible to all but one or two antibiotic classes [3]. In the face of such extensive levels of antibiotic resistance, clinicians have been forced to utilise colistin and polymyxin B (PMB) as last-line agents against XDR *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* that are capable of resisting carbapenems and most other agents [4,5]. However, the emergence of polymyxin heteroresistance and polymyxin-resistant strains has brought the utility of polymyxin monotherapies into question [6,7].

In response to the global decline in polymyxin susceptibilities, clinicians may be tempted to simply increase the dose of a polymyxin to maximise bacterial killing. Unfortunately, polymyxins are highly nephrotoxic agents and the likeliness of renal impairment has been associated with the daily dose of a polymyxin [8,9]. Given the narrow therapeutic indices of polymyxins, a strategy for overcoming attenuated polymyxin susceptibility without increasing polymyxin exposure is the use of polymyxins in combination with other agents. Polymyxins have a unique mechanism of action that involves disruption of the outer membrane integrity of Gram-negative bacteria, which may enhance the activity of other antibiotic classes [1,10]. Despite promising in vitro results, the usefulness of synergistic polymyxin combinations in the clinical setting remains controversial [11,12].

2. Methodology

This review covers recent studies that examined the in vitro and in vivo synergy of polymyxin combinations and evaluates whether any clinical evidence exists to validate the translation of preclinical work into human patients. Studies were retrieved using the search terms 'colistin combination' or 'polymyxin combination' in PubMed, with an emphasis on manuscripts published after 2010. In vitro studies were restricted to more advanced measures of bacterial killing such as time–killing investigations and dynamic models, whereas studies involving minimum inhibitory concentration (MIC) testing, chequerboard synergy and Etest methods were not examined. After consolidating all of the literature, manuscripts were chosen that epitomised recent in vitro, in vivo and clinical studies. To maintain consistency, synergy will be defined as a ≥2 log reduction in bacterial counts compared with reductions achieved by individual agents at any time during an experiment unless a time point is specified.

3. *Pseudomonas aeruginosa*

Several in vitro studies that utilised static antibiotic concentrations of polymyxins in combination with an aminoglycoside have recently been published. An investigation of *P. aeruginosa* biofilms found that colistin and tobramycin at 2× their respective MICs ($MIC_{colistin}$ = 2 mg/L; $MIC_{tobramycin}$ = 1 mg/L) separately reduced bacterial counts of a single *P. aeruginosa* strain to 4.59 $\log_{10}$ CFU/mL and 4.85 $\log_{10}$ CFU/mL after 24 h from a 7.95 $\log_{10}$ CFU/mL starting inoculum, whereas the combination of both agents resulted in a 24-h count of 2.60 $\log_{10}$ CFU/mL [13]. Another study investigated the activity of PMB (2 mg/L), rifampicin (2 mg/L), meropenem (64 mg/L) and amikacin (80 mg/L), alone and in combination, against 22

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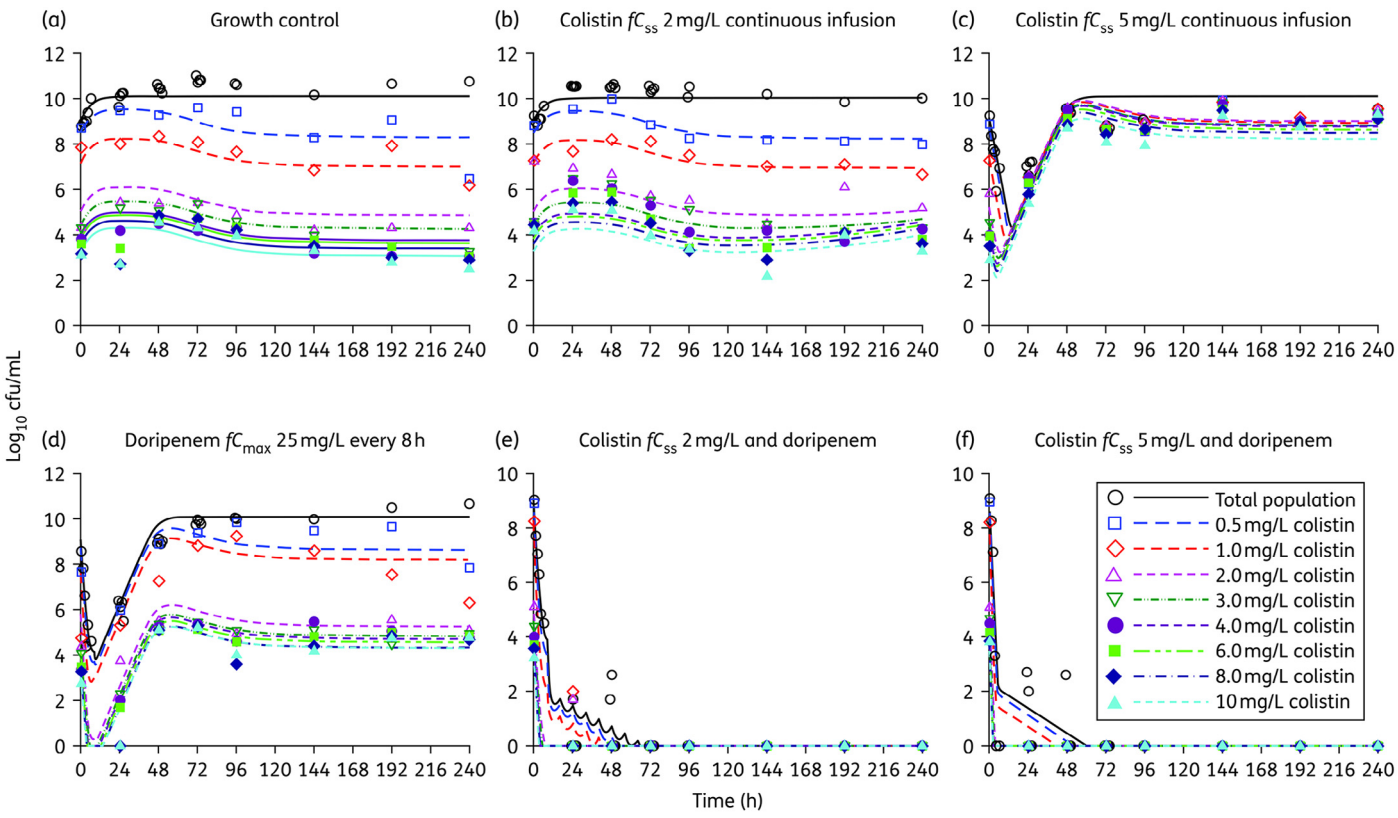

Fig. 1. Viable counts from a 240-h hollow-fibre infection model that examined the killing of colistin-heteroresistant *Pseudomonas aeruginosa* strain FADDI PA033 [colistin minimum inhibitory concentration (MIC) = 1 mg/L; doripenem MIC = 0.5 mg/L] at 10^9 CFU/mL [20]. Investigated antibiotic regimens included: (a) growth control; (b) colistin as a continuous infusion that achieved a constant free steady-state concentration (fC_{ss}) of 2 mg/L; (c) colistin given as a continuous infusion with a fC_{ss} of 5 mg/L; (d) doripenem given as a bolus dose with a free peak concentration (fC_{max}) of 25 mg/L every 8 h (q8h) with a simulated half-life of 1.5 h; (e) colistin given as a continuous infusion with a fC_{ss} of 2 mg/L and doripenem given as a bolus dose with fC_{max} of 25 mg/L q8h; and (f) colistin given as a continuous infusion with a fC_{ss} of 5 mg/L and doripenem given as a bolus dose with a fC_{max} of 25 mg/L q8h. In the key, the total population represents viable counts on antibiotic-free agar plates, and colistin concentrations represent the viable counts on colistin-containing agar plates. Symbols represent the observed counts and lines are the fits based on mathematical modelling. Reproduced with permission from Tsuji et al [20].

clinical XDR *P. aeruginosa* isolates collected in Singapore [14]. After 24 h of antibiotic exposure, none of the single agents achieved a 3 log reduction in bacterial counts, whereas the combination of meropenem + PMB resulted in ≥3 log reduction in 8/22 strains, and the triple combination of PMB + amikacin + rifampicin (or meropenem) achieved ≥3 log reduction in an additional 7 strains (6 strains for meropenem).

Another static time–killing experiment evaluated the potential synergy between fosfomycin (30, 150 or 300 mg/L) and PMB (0.5, 1 or 2 mg/L) against four clinical *P. aeruginosa* isolates and one reference strain at 10^6 CFU/mL [15]. In 24-h experiments, synergistic killing was achieved at fosfomycin concentrations ≥30 mg/L and at PMB concentrations ≥1 mg/L against two isolates susceptible to both antibiotics. In contrast, synergistic killing was most evident at fosfomycin concentrations ≥150 mg/L for the polymyxin-resistant strain, and synergy was only detected in one of two strains that were resistant to both antimicrobials.

Other static time–killing experiments investigated the killing of a polymyxin with a carbapenem. A study examining PMB in combination with doripenem against wild-type *P. aeruginosa* and hypermutator strains ($MIC_{polymyxin}$ = 1–2 mg/L) found that PMB concentrations up to 64 mg/L resulted in re-growth of all six stains at a 10^8 CFU/mL inoculum, whereas 4 mg/L PMB + 8 mg/L doripenem resulted in sustained killing up to 48 h [16]. Similarly, a study investigating colistin + imipenem in clinically relevant concentration arrays observed that several combinations of colistin + imipenem achieved synergy against colistin-resistant and imipenem-resistant *P. aeruginosa* isolates over 48 h both at 10^6 CFU/mL and 10^8 CFU/mL inocula [17].

The combination of a polymyxin and a carbapenem has also been investigated using dynamic in vitro models. In a dynamic biofilm model that utilised three colistin-susceptible *P. aeruginosa* isolates with varying doripenem susceptibilities ($MIC_{doripenem}$ = 0.125–128 mg/L), the combination of doripenem [peak concentration (C_{max}) = 25 mg/L] + colistin (constant at 3.5 mg/L) achieved ≥1–2 log of additional killing by 4 h compared with either agent alone, and the enhanced killing was sustained up to 72 h in all three strains [18]. A separate investigation used a one-compartment model to investigate doripenem (C_{max} = 2.5 mg/L or 25 mg/L) + colistin (constant at 0.5 mg/L or 2 mg/L) against a colistin-heteroresistant strain and a colistin-resistant MDR *P. aeruginosa* strain and observed that combinations of doripenem + colistin were capable of synergistic killing over 96 h against both stains at 10^6 CFU/mL and 10^8 CFU/mL [19]. Lastly, a 10-day hollow-fibre infection model was used to examine the activity of colistin (constant at 2 mg/L or 5 mg/L) + doripenem (C_{max} = 25 mg/L) against two colistin-heteroresistant strains and one colistin-resistant isolate with an initial inoculum of 10^9 CFU/mL (Fig. 1) [20]. Both colistin-heteroresistant strains were eradicated by colistin + doripenem by 72 h (monotherapies regrew), and although combination treatment was unable to eradicate the colistin-resistant strain, colistin + doripenem achieved >4 log reduction compared with either agent alone by 72 h.

In summary, the use of an aminoglycoside, fosfomycin or a carbapenem in conjunction with a polymyxin was able to confer