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Short Communication

Log reduction of multidrug-resistant Gram-negative bacteria by the neutrophil-derived recombinant bactericidal/permeability-increasing protein☆

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ABSTRACT

Multidrug-resistant (MDR) Gram-negative bacterial infections are a serious and ever-increasing threat for which limited therapeutic options exist. The bactericidal/permeability-increasing protein (BPI) is a cationic, neutrophil-derived, lipopolysaccharide (LPS)-binding protein that binds to Gram-negative bacteria (GNB) and LPS via its lipid A region. A recombinant fragment, rBPI-21, was studied extensively in clinical trials for meningococcal disease in the 1990s and exhibited no significant safety issues. In this report, a dose-dependent 1–2 log reduction of MDR *Pseudomonas* and *Acinetobacter* after 1 h incubation with rBPI-21 using clinically achievable doses is described. Given the dearth of novel antimicrobials expected to emerge from the pharmaceutical pipeline in the near future, exploration of rBPI-21 to combat MDR GNB is now warranted.

1. Introduction

Infections caused by multidrug-resistant (MDR) Gram-negative pathogens are a serious problem worldwide that show no sign of abating. In some areas of the USA, MDR Gram-negative organisms are endemic and require routine use of the polymyxin class of antibiotics (colistin and polymyxin B) as the sole therapeutic option. However, for many critically ill patients, use of the polymyxins is limited by renal toxicity. Most concerning are pan-resistant strains with minimum inhibitory concentrations (MICs) above achievable serum levels of the polymyxins, representing organisms for which no clinical therapeutic option exists. Whilst the development of new antibiotics with activity against MDR Gram-negative bacteria (GNB) is ongoing, all candidate drugs in phase 2 or 3 trials are derivatives of existing antibiotics, against which bacteria develop resistance relatively quickly [1]. Therefore, the existence of highly resistant GNB combined with the lack of truly novel antibiotics against GNB may portend the emergence of untreatable infections and herald a return to the pre-antibiotic era for these organisms [1].

The bactericidal/permeability-increasing protein (BPI) is an amphipathic, cationic, 50-kDa protein stored in the primary granules of neutrophils [2]. Bacteria encounter BPI in the extracellular space after neutrophil degranulation or intracellularly upon granule fusion with phagosomes. BPI is a two-domain molecule (Fig. 1), with a cluster of positively charged amino acids at its N-terminus [2]. BPI's selectivity and high affinity for GNB arises from the interaction of its positively charged N-terminus with the negatively charged lipid A portion of lipopolysaccharide (LPS) [2]. Given the relatively conserved nature of lipid A, the activities of BPI extend to many GNB including *Escherichia coli*, *Pseudomonas aeruginosa* [2], *Acinetobacter baumannii* [5], *Neisseria* [6] and *Campylobacter* [7]; it is variably active against *Klebsiella pneumoniae* [8] but is inactive against other GNB such as *Serratia* [8] and *Enterobacter* [8].

The antibacterial and LPS-neutralising activities of BPI are entirely attributable to its highly cationic, ca. 21-kDa N-terminal half known in recombinant form as rBPI-21 [2] (Fig. 1). This was developed by XOMA Corp. (Berkeley, CA) as opebecan in the 1990s for the treatment of meningococcaemia and did not show any significant safety issues [9]. Although there was a trend towards increased survival and clinically meaningful improvements in morbidities in the rBPI-21 + conventional treatment group versus the conventional treatment group [9], the phase 3 trial was underpowered due to the rarity of this infection, and rBPI-21 did not gain US Food and Drug Administration (FDA) approval for treatment of meningococcaemia.

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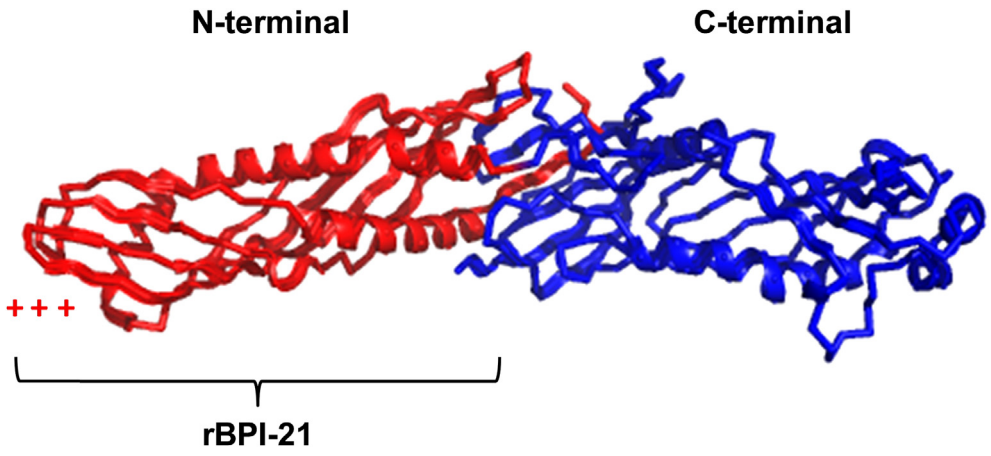

Fig. 1. Crystal structure of bactericidal/permeability-increasing protein (BPI) [3] reveals an elongated, two-domain protein in which 18 positively charged amino acids (indicated by red plus signs) are clustered at the N-terminus. The N-terminal half of BPI (shown in red), corresponding to amino acids 1–193, contributes the antibacterial and lipopolysaccharide (LPS)-neutralising properties of the holoprotein and corresponds to the recombinant molecule rBPI-21 [4]. The C-terminal half (shown in blue, corresponding to amino acids 194–456) contributes opsonic and LPS delivery properties [10]. The graph was adapted from Fig. 1A in [3]. (For interpretation of colour in the artwork, the reader is referred to the web version of the article.)

Since then, the emergence of serious infections caused by MDR strains of GNB has necessitated the use of nephrotoxic drugs such as the polymyxins, or bacteriostatic drugs such as tigecycline. Notably, the latter is not FDA-approved to treat bacteraemia and has no activity against *Pseudomonas*. In the direst clinical situations, the bacteria manifest resistance to all available drugs including the polymyxins. Given that lipid A is relatively well conserved and its recognition underlies BPIs cytotoxicity against a wide range of GNB, it was hypothesised that rBPI-21 would also affect killing of MDR and pan-resistant Gram-negative bacterial clinical isolates. To test this hypothesis, the log reduction of several drug-resistant clinical isolates of *A. baumannii*, *P. aeruginosa* and *K. pneumoniae* following exposure to increasing doses of rBPI-21 was measured.

2. Materials and methods

2.1. Protein

rBPI-21 (XOMA Corp.) was the generous gift of Dr Jerrold Weiss (University of Iowa, Des Moines, IA) and was maintained in 10 mM sodium acetate buffer (pH 4.0).

2.2. Bacteria

Escherichia coli K1/r is an encapsulated, short-chain LPS laboratory strain with well-defined susceptibility to BPI [10]. Clinical isolates of *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* are described in Table 1. Overnight cultures were grown in Luria broth (Becton Dickinson, Sparks, MD) at 37 °C in 5% CO_2 with shaking (120 rpm). Subcultures were prepared by 1:100 dilution of the overnight cultures in fresh broth and were grown for 2.5–3.5 h to mid/late log phase based on previously measured growth curves (data not shown).

2.3. Antibiotic susceptibility testing

The MICs of extended-spectrum β-lactams and carbapenems for all strains were determined by VITEK® 2 (bioMérieux, Durham, NC), as was the MIC of tigecycline against *A. baumannii* 43. The MICs of tigecycline against *A. baumannii* 3 and all of the *K. pneumoniae* strains, and of polymyxin B against all strains, were determined by Etest (bioMérieux). Tigecycline was not tested against *P. aeruginosa* as this drug is not clinically active against it. Susceptibility and resistance interpretations of the polymyxin B MICs for *A. baumannii* and *P. aeruginosa* were based on breakpoints established by the Clinical and Laboratory Standards Institute (CLSI). Since there are currently no CLSI breakpoints established for tigecycline against *K. pneumoniae*, the FDA-approved breakpoints in the manufacturer's prescribing information were used. Also, there are no CLSI breakpoints for polymyxin B against *K. pneumoniae*; therefore, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints were used. EUCAST breakpoints were also used for *A. baumannii* and tigecycline.

2.4. Antibacterial assay

Bacteria were sedimented at 300 × *g* for 5 min and were resuspended in sterile Hank's Balanced Salt solution buffered with 10 mM HEPES at pH 7.4 (both Invitrogen, Carlsbad, CA) and 0.05% human serum albumin followed by the addition of rBPI-21 or sodium acetate buffer to a final bacterial concentration of 10^6 CFU/mL and final rBPI-21 concentrations of 10, 30 and 90 nM. The rBPI-21 stock concentration was 95 μM, requiring a 1058-fold dilution to achieve the maximal final concentration of 90 nM; the sodium acetate was similarly diluted for the negative control. Bacterial concentrations were determined by measuring absorbance at 595 nm (A_{595}) using a NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE) and were confirmed by plating aliquots immediately before the experiment. The suspensions were incubated at 37 °C for 1 h in 5% CO_2 with shaking (120 rpm). The samples were diluted 1000-fold to minimise rBPI-21 carry-over and aliquots were plated on 5% sheep blood agar plates (Becton Dickinson) and incubated overnight at 37 °C in 5% CO_2. Bacterial killing was quantitated by enumeration of CFU. Log_{10} killing was calculated by comparison with the 0 nM rBPI-21 (sodium acetate only) condition. The limit of detection was 3×10^3 CFU.

2.5. Statistical testing

Significance was calculated using the Student's one-tailed *t*-test, with a *P*-value of <0.01 regarded as significant.

3. Results

After 1 h incubation of the *E. coli* control strain K1/r with 10, 30 and 90 nM rBPI-21, a 1.8, 2.3 and 3.2 log reduction, respectively,