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Potentiation of Antibiotic Activity by a Novel Cationic Peptide, SPR741

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ABSTRACT

Objective: Novel approaches to the treatment of multi-drug resistant (MDR) Gram negative bacterial (GNB) infections are urgently required. One approach is to potentiate the efficacy of existing antibiotics whose spectrum of activity is limited by the permeability barrier presented by the GNB outer membrane (OM). Cationic peptides derived from polymyxin B have been used to permeabilise the OM of GNB, granting antibiotics that would otherwise be excluded access to their targets. We assessed the *in vitro* efficacy of combinations of SPR741 with conventional antibiotics against *Escherichia coli* (*Ec*), *Klebsiella pneumoniae* (*Kp*), and *Acinetobacter baumannii* (*Ab*).

Methods: Efficacy was assessed in checkerboard assays. The minimum inhibitory concentration (MIC) of SPR741, antibiotics, and combinations thereof was defined as the lowest concentration that inhibited growth of *Ec* ATCC 25922, *Ab* NCTC 12156 and *Kp* ATCC 43816. *Ec* BW25113, *ΔtolC::kan* and *ΔacrA::kan* were used to assess the contribution of the multi drug efflux pump AcrAB-TolC to susceptibility to the combinations. Interactions were assessed by calculating fractional inhibitory concentration indices (FICI) for each combination in which the MIC differed from compounds in isolation. Interactions were defined as: FICI > 4, antagonism; 0.5-4, no interaction; <0.5, synergy. The minimum bactericidal concentration of combinations in the presence of 5% surfactant (Survanta) was also determined.

Results (updated): Of 35 antibiotics tested, the MIC of 8 – azithromycin, clarithromycin (CLR), erythromycin (ERY), fusidic acid (FA), mupirocin, retapamulin (RET) rifampicin (RIF), telithromycin - against *Ec* and *Kp* was reduced 32 - 8,000-fold in the presence of 8-16 µg/mL SPR741; against *Ab*, similar potentiation was achieved with CLR, ERY, FA, and RIF. SPR741 was able to potentiate antibiotics that are substrates of AcrAB-TolC, effectively circumventing the pump's contribution to intrinsic antibiotic resistance. RIF in combination with SPR741 were bactericidal at concentrations similar to the MIC irrespective of the presence of Survanta.

Conclusion: The efficacy of several antibiotics with diverse targets was substantially increased when combined with SPR741. These studies support the development of SPR741 in combination with antibiotics for treatment of MDR GNB.

INTRODUCTION

The emergence of Gram negative bacteria with increasingly multi-drug resistant profiles has become a serious public health concern worldwide, with isolates commonly reported resistant to carbapenems. However, no totally new class of antibiotics active against Gram negative bacteria has been introduced for over 40 years.

One of the most significant barriers to the development of novel anti-Gram negative agents is the bacterial outer membrane. One novel therapeutic approach is to combine an antibiotic with a “potentiator” molecule that permeabilises the outer membrane, granting antibiotics that would otherwise be ineffective, access to their targets. SPR741, a cationic peptide derived from polymyxin B, is one such potentiator molecule that is under development for the treatment of serious Gram negative bacterial infections. Here, we show that SPR741 substantially enhances the *in vitro* efficacy of several conventional antibiotics against *E. coli*, *K. pneumoniae*, and *A. baumannii*.

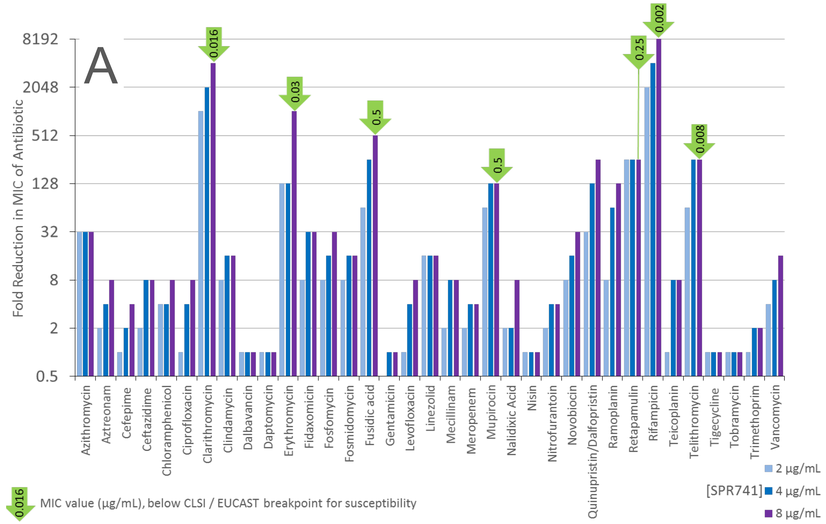
METHODS

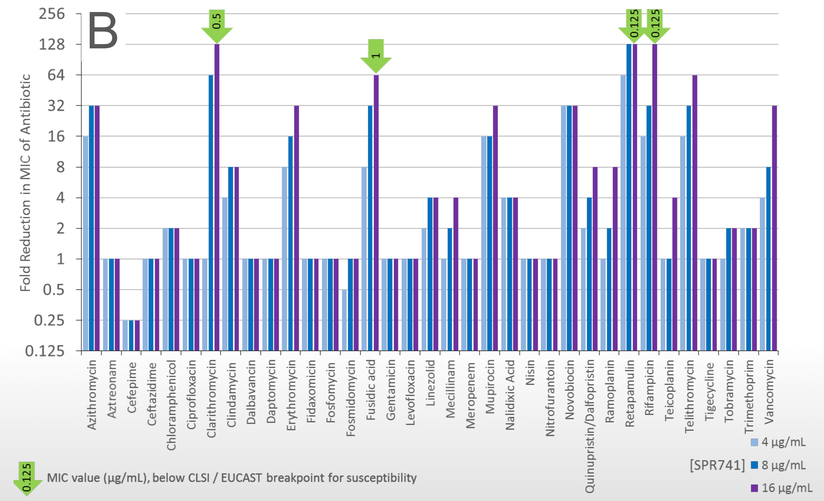
The MIC of SPR741 and antibiotics was determined in cation-adjusted Mueller-Hinton broth, using Clinical and Laboratory Standards Institute (CLSI) guidelines M7-A10 & M100-S26. The MIC of combinations was determined in a checkerboard format based on CLSI M7-A10 and the Clinical Microbiology Procedures Handbook, 3rd Edition (Chapter 5.12, ASM Press, Washington DC, 2010). Sterile, flat-bottomed polystyrene plates (Corning 3370) and an assay volume of 200 µL were used throughout. The MIC of SPR741, antibiotics, and combinations thereof was defined as the lowest concentration that inhibited visible bacterial growth. Minimum bactericidal concentrations (MBCs) were inferred by adding 0.01% resazurin to completed assays and measuring absorbance at 570nm; the lowest concentration of test articles resulting in no change in absorbance was defined as the MBC.

Results are reported as the maximum fold-reduction in MIC of each antibiotic at three concentrations of SPR741 for *E. coli* ATCC 25922 (2, 4, and 8 µg/mL) or *K. pneumoniae* ATCC 43816 and *A. baumannii* NCTC 12156 (4, 8, and 16 µg/mL SPR741).

RESULTS

Figure 1. SPR741 potentiates the activity of antibiotics against *E. coli* ATCC 25922 (A), *K. penumoniae* ATCC 43816 (B) and *A. baumannii* NCTC 12156 (C). The fold reduction in the MIC of each of 35 antibiotics is displayed at each of three concentrations of the potentiator (2, 4, or 8 µg/mL [A]; 4, 8, or 16 µg/mL [B and C]). Green arrows indicate that the MIC of the antibiotic has been reduced to, or below, the CLSI or EUCAST breakpoint for susceptibility for this organism/antibiotic combination, or where this information is not available, below the equivalent breakpoint or ECOFF for *Staphylococcus* spp.





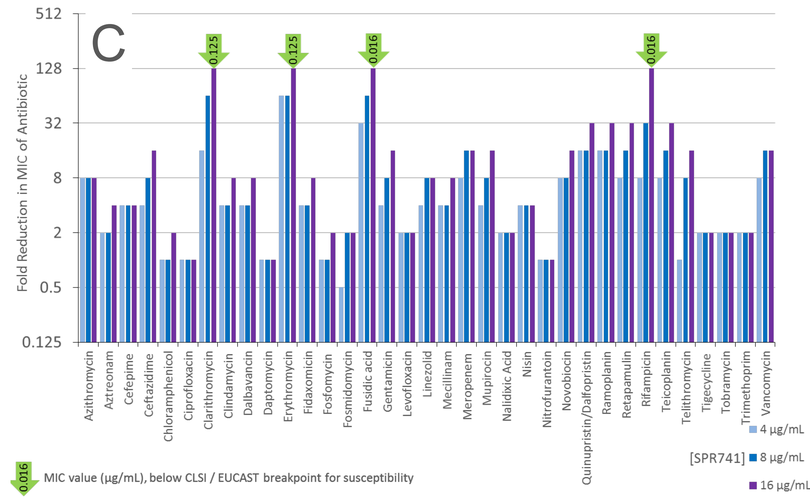


Figure 2. SPR741 potentiates the activity of antibiotics that are substrates of the AcrAB-TolC multi-drug efflux pump. The MICs (µg/mL) of clarithromycin, mupirocin and retapamulin were determined against *E. coli* BW25113 and its *ΔtolC::kan* and *ΔacrA::kan* derivatives. Horizontal lines indicate the susceptibility breakpoint/ECOFF of each antibiotic for *Staphylococcus* spp.

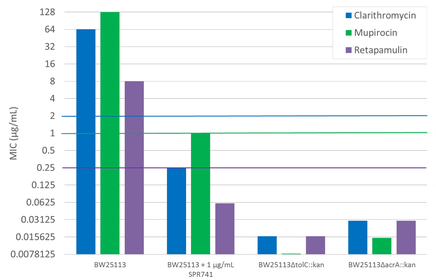
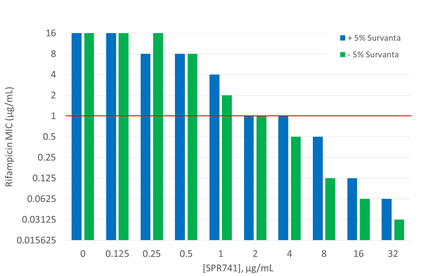


Figure 3. Potentiation by SPR741 is not impaired in the presence of the surfactant Survanta. The MBC (µg/mL) of combinations of rifampicin and SPR741 was determined against *K. pneumoniae* ATCC 43816 in the presence (blue bars) or absence (green bars) of 5% Survanta. Horizontal red line indicates the susceptibility breakpoint of rifampicin for *Staphylococcus* spp.



CONCLUSION

- SPR741 can potentiate the activity of antibiotics with diverse mechanisms of action that are normally ineffective against Gram negative bacteria.
- In combination with SPR741, the MIC of clarithromycin, fusidic acid, and rifampicin against *E. coli* ATCC 25922, *K. pneumomiae* ATCC 43816 and *A. baumannii* NCTC 12156 can be reduced to levels that should render them susceptible to treatment.
- Combination with SPR741 also resulted in substantial (>32-fold) reductions in the MIC of azithromycin, erythromycin, mupirocin, retapamulin, and telithromycin against *E. coli* and *K. pneumoniae*
- Efficacy of the rifampicin/SPR741 combination was not reduced in the presence of 5% Survanta