Potentiation of Antibiotic Activity by a Novel Cationic Peptide, SPR741

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ABSTRACT

Objective: Novel approaches in 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 barriers presented by the GNB outer membrane. Cationic peptides derived from polyiminobutyric acid (PIB) have been shown in preliminary studies to permeabilize 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 (E. coli), Klebsiella pneumoniae (K. pneumoniae), and Acinetobacter baumannii (A. baumannii).

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 E. coli ATCC 25922, A. baumannii TCC 43816 and K. pneumoniae NCTC 12156. 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 (MBC) was inferred by visible bacterial growth. Minimum bactericidal concentrations (MBCs) were inferred by visible bacterial growth. Minimum inhibitory concentrations (MICS) were inferred from compound dilution plates.

Results: Of 36 antibiotics tested, the MIC of SPR741 was reduced 32-8,000-fold in the presence of 12-16 µg/mL SPR741 against E. coli and K. pneumoniae. Inhibition was achieved in combination with clarithromycin (CLR), erythromycin (ERY), mupirocin, retapamulin, telithromycin, rifampicin (RIF), and fusidic acid. MBCs of SPR741 were bactericidal at concentrations similar to the MIC irrespective of the presence of 5% 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 permeabilizes the outer membrane, granting antibiotics that would otherwise be ineffective, access to their targets. SPR741, a cationic peptide derived from polyiminobutyric acid 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 MT-41A & M100-S26. The MIC of combinations was determined in a checkerboard format based on CLSI MT-41A and the Clinical Microbiology Procedures Handbook, 3rd Edition (Chapter 5.12, ASM Press, Washington DC, 2010). Sterile, flat-bottomed polystyrene plates (Corning, 1 TCM) 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 from compound dilution plates. Minimum bactericidal concentrations (MBCs) were inferred from compound dilution plates. Minimum inhibitory concentrations (MICS) were inferred from compound dilution plates.

Results: Of 35 antibiotics tested, the MIC of 8 – azithromycin, clarithromycin (CLR), erythromycin (ERY), mupirocin, retapamulin, telithromycin, rifampicin (RIF) and fusidic acid, and rifampicin against E. coli ATCC 25922, K. pneumoniae ATCC 43816 and A. baumannii NCTC 12156 can be reduced to levels that should render them susceptible to treatment.

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. pneumoniae 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.

Figure 1. SPR741 potentiates the activity of antibiotics against E. coli ATCC 25922 (A), K. pneumoniae 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 of Survanta did not alter the MIC of SPR741.

Figure 2. SPR741 potentiates the activity of antibiotics that are substrates of the AcrAB-TolC multi-drug efflux pump. The MICs of clarithromycin, mupirocin and retapamulin were determined against wild-type E. coli ATCC 25922 and A. baumannii TCC 43816 in the presence (blue bars) or absence (green bars) of 5% Survanta. Horizontal red line indicates the susceptibility breakpoint of each antibiotic for Stephanocephalus spp.

Figure 3. Potentiation by SPR741 is not impaired in the presence of 5% Survanta. The MICs (µg/mL) of combinations of SPR741 and antibiotics were determined against K. pneumoniae ATCC 43816 in the presence of Survanta (green bars). Horizontal line indicates the susceptibility breakpoint of each antibiotic for Stephanocephalus spp.