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In Vitro Bactericidal Activity and Post-Antibiotic Effect of Tebipenem (SPR859) Against Susceptible and Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae as Compared to Levofloxacin (LVX) and Meropenem (MEM)

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ABSTRACT

• **Background:** Antimicrobial resistance among Gram-negative uropathogenic bacteria has increased in recent years. Tebipenem (SPR859) has potent activity against Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs). SPR994 (tebipenem-pivoxil, the orally active prodrug of SPR859) is being developed as a therapeutic for complicated urinary tract infections in adults. We evaluated the bactericidal activity and post-antibiotic effect (PAE) of SPR859 against target pathogens *in vitro*.

• **Methods:** Broth time-kill studies were performed on susceptible and ESBL+ isolates of *Escherichia coli* (Ec) ATCC 25922 and 35218, *Klebsiella pneumoniae* (Kp) ATCC 43816 and 700603, and *Proteus mirabilis* (Pm) ATCC 29906 and clinical isolate JY040, in accordance with CLSI M26-A. Log-phase cultures at ~1.0E+05 CFU/mL in cation-adjusted Mueller Hinton broth (CAMHB) were treated with antimicrobial agents at 2x - 8x MIC, viability was monitored for 24 h, and bactericidal activity was defined as a 3log10 reduction in CFU/mL of the initial inoculum.

• The *in vitro* PAE of SPR859 and comparators was established against Ec ATCC 25922 and Kp ATCC 43816. Log phase cultures were treated with antimicrobial agents at 4x and/or 8x MIC for 1 h, diluted 1:1000 into fresh CAMHB, and viability was monitored for 6 h by plating for CFU/mL. PAE = T – C where T and C are the time required to increase 1 $\log_{10}$ CFU following 1:1000 dilution for the bacteria treated with and without the agents, respectively.

• **Results:** SPR859 was bactericidal at 2x - 8x MIC within 4 h against Ec and Kp strains, comparable to MEM at 4x MIC. SPR859 was bactericidal against susceptible Pm ATCC 29906 at 2x MIC within 8 h, and at 4x – 8x MIC within 4 h; against ESBL+ Pm JY040 at 2x MIC within 4 h, and at 4x – 8x MIC within 2 h. MEM at 4x MIC was bactericidal against both Pm isolates within 2 h. LVX at 4x MIC was bactericidal against all isolates within 2 h. 4x – 8x MIC of SPR859 produced a negligible PAE against Ec ATCC 25922 and Kp ATCC 43816 with values of ≤0.4 h, comparable to MEM. LVX at 4x MIC showed a PAE of 0.8 – 2 h against Ec ATCC 25922 and 2.9 - 3.1 h against Kp ATCC 43816.

• **Conclusions:** SPR859 was bactericidal at 4x – 8x MIC against Ec, Kp, and Pm, and at 2x MIC against Ec and Kp strains within 4 h. SPR859 did not demonstrate a significant PAE against Ec and Kp. SPR859 was comparable to MEM (an IV administered carbapenem) in both time-kill and PAE studies, thus supporting the development of SPR994, the prodrug of SPR859, as a potential new oral option for ESBL+ Gram-negative infections.

INTRODUCTION

Antimicrobial resistance among Gram-negative uropathogenic bacteria has increased in recent years. Tebipenem (SPR859) has potent activity against Enterobacteriaceae producing extended-spectrum b-lactamases (ESBLs). SPR994 (tebipenem-pivoxil, the orally active prodrug of SPR859) is being developed as a therapeutic for complicated urinary tract infections in adults. We evaluated the bactericidal activity and post-antibiotic effect (PAE) of SPR859 against target pathogens *in vitro*.

METHODS

• Broth time-kill studies were performed on susceptible and ESBL+ isolates of *Escherichia coli* (Ec) ATCC 25922 and 35218, *Klebsiella pneumoniae* (Kp) ATCC 43816 and 700603, and *Proteus mirabilis* (Pm) ATCC 29906 and clinical isolate JY040, in accordance with CLSI M26-A[1]. Log-phase cultures at ~1.0E+05 CFU/mL in cation-adjusted Mueller Hinton broth (CAMHB) were treated with antimicrobial agents at 2x - 8x MIC as determined by CLSI M07-A10 methodology[2], viability was monitored for 24 h, and bactericidal activity was defined as a $3\log_{10}$ reduction in CFU/mL of the initial inoculum.

• The *in vitro* PAE of SPR859 and comparators was established against Ec ATCC 25922 and Kp ATCC 43816 using a method established in Antibiotics in Laboratory Medicine[3]. Log phase cultures were treated with antimicrobial agents at 4x and/or 8x MIC for 1 h, diluted 1:1000 into fresh CAMHB, and viability was monitored for 6 h by plating for CFU/mL. PAE = T – C where T and C are the time required to increase 1 $\log_{10}$ CFU following 1:1000 dilution for the bacteria treated with and without the agents, respectively.

RESULTS

Figure 1. Time-kill studies of SPR859, MEM and LVX

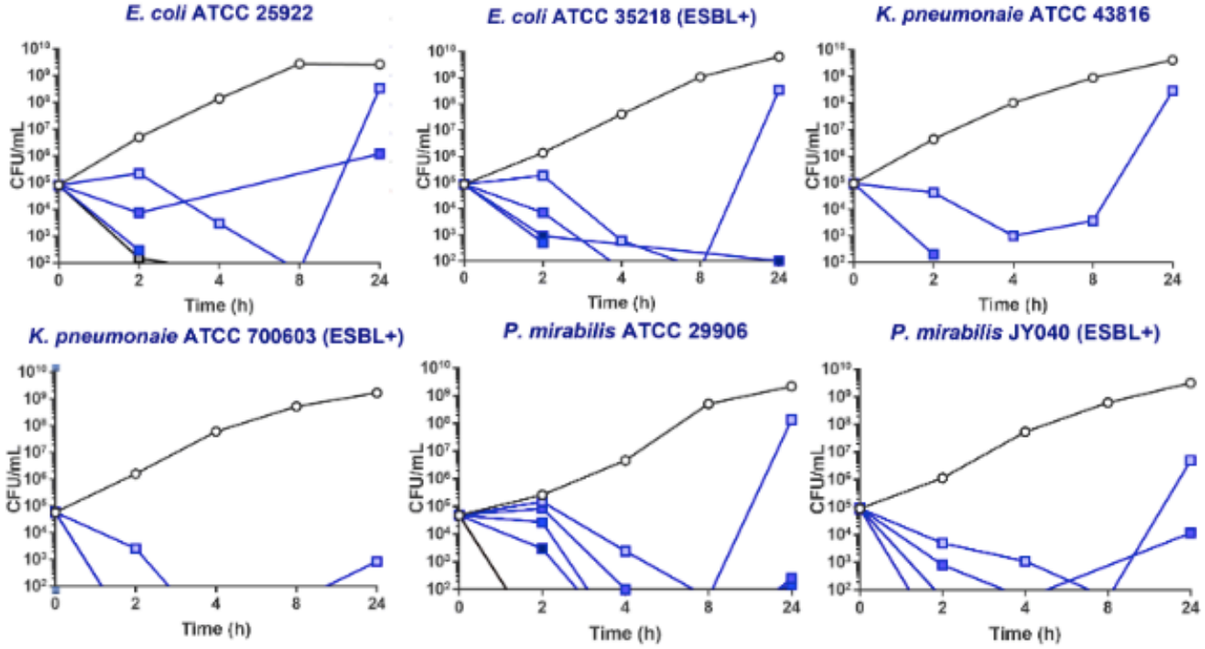
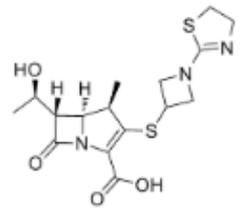


Figure 3. Tebipenem (SPR859)



• SPR859 was bactericidal at 2x - 8x MIC within 4 h against Ec and Kp strains, comparable to MEM at 4x MIC.

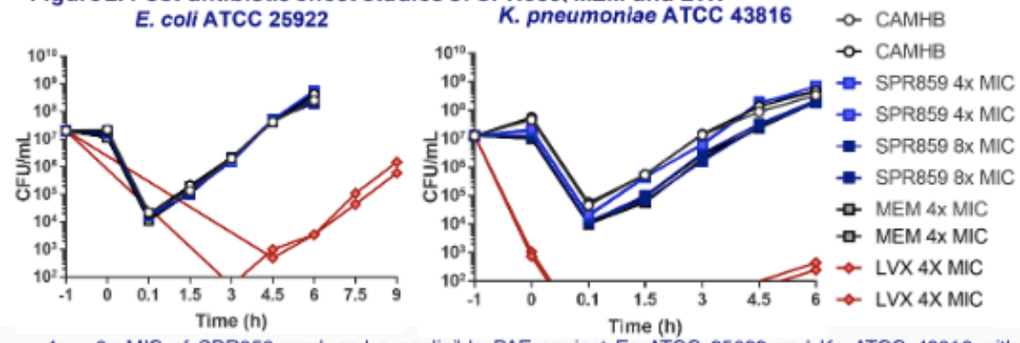
• SPR859 was bactericidal against susceptible Pm ATCC 29906 at 2x MIC within 8 h, and at 4x – 8x MIC within 4 h

• SPR859 was bactericidal against ESBL+ Pm JY040 at 2x MIC within 4 h, and at 4x – 8x MIC within 2 h.

• MEM at 4x MIC was bactericidal against both Pm isolates within 2 h.

• LVX at 4x MIC was bactericidal against all isolates within 2 h .

Figure 2. Post-antibiotic effect studies of SPR859, MEM and LVX



• 4x – 8x MIC of SPR859 produced a negligible PAE against Ec ATCC 25922 and Kp ATCC 43816 with values of ≤0.4 h, comparable to MEM.

• LVX at 4x MIC showed a PAE of 0.8 – 2 h against Ec ATCC 25922 and 2.9 - 3.1 h against Kp ATCC 43816.

CONCLUSIONS

• SPR859 was bactericidal at 4x – 8x MIC against Ec, Kp, and Pm, and at 2x MIC against Ec and Kp strains within 4 h.

• SPR859 did not demonstrate a significant PAE against Ec and Kp. SPR859 was comparable to MEM (an IV administered carbapenem) in both time-kill and PAE studies, thus supporting the development of SPR994, the prodrug of SPR859, as a potential new oral option for ESBL+ Gram-negative infections.

REFERENCES

[1] CLSI M26A Methods for Determining Bactericidal Activity

[2] CLSI M07-A10 Methods for Dilution Antimicrobial Susceptibility Testing for Aerobic Bacteria

[3] Antibiotics in Laboratory Medicine - 4th Edition