Bactericidal Activity of Piperacillin-Tazobactam in Combination with SPR741 Against Susceptible, Extended-Spectrum Beta-Lactamase Producing, and Multidrug Resistant Escherichia coli, Klebsiella pneumoniae, and Enterobacter aerogenes

Yanming Zou¹, N Cotroneo², T Listler², A Rubio²
¹HD Biosciences (China), Shanghai, P. R. China; ²Spero Therapeutics, Cambridge, Massachusetts, USA

ABSTRACT

Background: SPR741 is a novel polymer B derivative with minimal intrinsic antimicrobial activity and reduced nephrotoxicity. SPR741 interacts with the outer membrane of gram-negative (G-N) bacteria, forming permeable channels of colo-administered antimicrobial compounds such as piperacillin-tazobactam (TZP). Materials and Methods: The bactericidal activity of TZP with or without 8 mg/L SPR741 was assessed against 36 isolates of Escherichia coli, Klebsiella pneumoniae, and Enterobacter aerogenes. TZP susceptible, multidrug-resistant, and extended-spectrum beta-lactamase (ESBL)-producing isolates (K. pneumoniae, E. coli, and K. pneumoniae) were also assessed against a CLSI broth microdilution method. Results: Multiple MICs at 8 mg/L reduced the MICs of HUGG TZP susceptible isolates by 0.5-8 fold. SPR741 alone showed 67% reduction in MICs of 79% of TZP-resistant isolates from 16 mg/L to 4 mg/l, while the aztreonam-resistant isolates were significantly reduced. SPR741 showed 2-4 fold reductions in the MICs of TZP-resistant isolates, with a peak reduction of 8 mg/L to 2 mg/l. SPR741 in combination with TZP demonstrated a 3-5 fold MIC reduction in susceptible E. coli and K. pneumoniae, as well as susceptible and ESBL-positive K. pneumoniae at 4 mg/l, respectively. SPR741 at 8 mg/L was observed for the resistant isolates. Conclusion: These results illustrate that SPR741 significantly enhances the antimicrobial activity and stability of TZP against clinical isolates of Enterobacteriaceae. These data support the use of SPR741 in combination with this SOC agent.

INTRODUCTION

Resistance to gram-negative bacteria is a growing threat and has impacted the utility of SOC agents, especially in high-risk populations. SPR741 is a novel cationic polymer B derivative with minimal intrinsic antimicrobial activity and reduced nephrotoxicity. SPR741 interacts with the outer membrane of gram-negative (G-N) bacteria, compromising the integrity of the Lipo polysaccharide (LPS) barrier, thus enhancing penetration and activity of antimicrobial compounds such as piperacillin-tazobactam (TZP) we co-administered.

METHODS

- The potency and bactericidal activity of TZP alone and in combination with SPR741 (0.5 mg/L) was assessed in vitro vs. 36 clinically relevant susceptible, multidrug-resistant (MDR) and TZP-resistant isolates of E. coli, K. pneumoniae, and Enterobacter aerogenes using CLSI method M7-A10 to determine brainstorm, minimal inhibitory concentrations (MICs). 
- The bactericidal activity and the extended-spectrum beta-lactamase (ESBL) producing isolates of E. coli and K. pneumoniae were also assessed using the CLSI broth microdilution method. In brief, cultures were grown in log phase in CAMHB, diluted to ~0.5-65 CFU/mL, and incubated at 36°C for 24 hr. 

RESULTS

- SPR741 reduced the MICs of 19/30 TZP-resistant isolates by 4-95 fold using the CLSI breakpoint of 16 mg/L.
- SPR741 reduced the MICs of 79% of TZP-resistant isolates from 16 mg/L to 4 mg/l, into the susceptible range, with an MIC/MIC ratio of 1 was observed for the resistant isolates.
- TZP in combination with SPR741 was bactericidal at 1-4x MIC against 10/16 E. coli, 9/10 K. pneumoniae, and 8/10 Enterobacter aerogenes.

CONCLUSIONS

These data illustrate that SPR741 significantly enhances the antimicrobial activity and stability of TZP against clinically relevant isolates of Enterobacteriaceae, and represents a promising new treatment option for infections caused by TZP susceptible and non-susceptible bacterial isolates.

REFERENCES

- This work was partially supported by the National Institutes of Health and the National Science Foundation grant number 2014-18489.

ACKNOWLEDGMENTS

- This presentation is supported by the Cooperative Agreement Number 5U90/0024617-04 from ASHP Foundation and by an academic year fellowship from Spero Pharmaceuticals, as determined by the investigators. The contents are solely the responsibility of the authors and do not necessarily represent the official views of ASHP or Spero Pharmaceuticals.

Figure 1. Killing kinetics profiles of SPR741, TZP, MDM, and SPR741/TZP combinations at various concentrations

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC</th>
<th>W2/L (μg/mL)</th>
<th>MIC/MIC</th>
<th>MIC/MIC ratio</th>
<th>W2/L (μg/mL)</th>
<th>MIC/MIC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>4</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>8</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>E. coli</td>
<td>4</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>8</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>E. coli</td>
<td>4</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>8</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 2. Structure of SPR741

- SPR741 reduced the MICs of 19/30 TZP-resistant isolates by 4-95 fold using the CLSI breakpoint of 16 mg/L.
- SPR741 reduced the MICs of 79% of TZP-resistant isolates from 16 mg/L to 4 mg/l, into the susceptible range, with an MIC/MIC ratio of 1 was observed for the resistant isolates.
- TZP in combination with SPR741 was bactericidal at 1-4x MIC against 10/16 E. coli, 9/10 K. pneumoniae, and 8/10 Enterobacter aerogenes.

Figure 3. Structure of SPR741

- TZP in combination with SPR741 demonstrated a 30-40 fold MIC reduction in susceptible, ESBL, and TZP-resistant E. coli as well as susceptible and ESBL-positive K. pneumoniae at 4x MIC within four to eight hours.
- TZP alone at 4x MIC achieved a similar effect within 8 hours against the same strain.
- Results were similar to TZP alone, but at a significantly lower concentration of SPR741.

Figure 4. Structure of SPR741