A Pharmacokinetic-Pharmacodynamic Evaluation of the Novel Antibiotic Potentiator, SPR741, in Combination with Piperacillin/Tazobactam Against Enterobacteriaceae

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INTRODUCTION

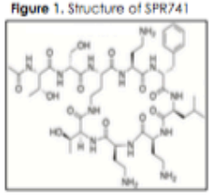
- Antimicrobial resistance has become one of the largest threats to global health.
- SPR741, a novel polymyxin B derivative with minimal intrinsic antibacterial activity and reduced non-clinical nephrotoxicity, acts as a potentiator when administered in combination with antibiotics with activity against Gram-negative pathogens.
- Herein, we describe a series of 24-hour one-compartment *in vitro* studies designed to determine the SPR741 exposure required to restore activity of a standard clinical dose of piperacillin/tazobactam (PIP/TAZ) in the treatment of resistant organisms.

METHODS

Antimicrobial Agents and Challenge Isolates

- SPR741 (**Figure 1**) was provided by Spero Therapeutics (Cambridge, MA). Piperacillin and tazobactam were purchased from Sigma Aldrich (St. Louis, MO).
- A panel of seven Enterobacteriaceae isolates known to produce a variety of β-lactamase enzymes was selected from the antibiotic resistance bank provided by the Centers for Disease Control (Atlanta, GA) or purchased from American Type Culture Collection (Manassas, VA), based upon resistance to PIP/TAZ (MIC values ≥32 mg/L).

Figure 1. Structure of SPR741



Susceptibility Testing

- Minimum inhibitory concentrations (MIC) were determined by ICPD and JMI laboratories for the panel of isolates following Clinical Laboratory Standards Institute guidelines [1].
  - MIC values were determined for piperacillin and SPR741 alone, piperacillin in combination with a fixed value of 4 mg/L of tazobactam (PIP/TAZ) and PIP/TAZ in combination with SPR741 at fixed values of 4 and 8 mg/L or at fixed ratios of PIP/TAZ to SPR741 (1:2, 1:1 and 2:1).
  - All MIC values are presented as the modal value.

One-Compartment *in Vitro* Infection Model

- A one-compartment *in vitro* infection model consisting of a central infection compartment containing Mueller-Hinton broth, the challenge isolate, and a magnetic stir bar to ensure homogeneity, was placed in an incubator set at 35°C.
- Drug-free medium was pumped into the central compartment via peristaltic pumps in order to simulate human PIP/TAZ and SPR741 free-drug concentration time-profiles.
- An initial inoculum of 1.0×10^6 colony forming unit (CFU)/mL was utilized for each isolate.
  - Samples for bacterial enumeration were washed twice with normal saline, serially diluted, and cultured on drug-free agar plates.
  - Drug concentrations were assessed by a qualified liquid chromatography mass-spectrometry method.

Dose-Ranging Studies

- A series of 24-hour dose-ranging studies were completed in which the PIP/TAZ clinical dosing regimen was administered alone and in combination with a range of SPR741 exposures.
  - All isolates were exposed to PIP/TAZ 4.5 g dose administered as a 1-hour infusion every 8 hours (q8h), simulated using a 1 hour half-life and assuming 30% protein binding, based on the package insert [2].
  - Total SPR741 area under the concentration-time curve (AUC) values administered q8h, ranged from 8.53 to 281 mg•h/L, simulated using a 2.6 hour half-life and 70% protein binding based on single ascending dose studies in healthy volunteers [Data on File].

METHODS

Pharmacokinetic-Pharmacodynamic Analysis

- Data from the dose-ranging studies were pooled and evaluated using Hill-type models and non-linear least squares regression.
- The relationship between change in $\log_{10}$ CFU/mL from baseline at 24 hours and the free-drug ratio of the area under the concentration-time curve to the MIC (AUC:MIC) based on the SPR741 AUC value indexed to the MIC value of each challenge isolate as described in the susceptibility studies, was evaluated.
  - Once the pharmacokinetic-pharmacodynamic (PK-PD) relationship that best described the activity of SPR741 in combination with PIP/TAZ was identified, the SPR741 free-drug AUC:MIC ratio associated with net bacterial stasis and a 1-$\log_{10}$ CFU/mL reduction from baseline was determined.

RESULTS

Susceptibility Testing

- As shown in **Table 1**, the MIC values for piperacillin alone, PIP/TAZ, and SPR741 alone ranged from 32 to >256 mg/L.
- When PIP/TAZ was evaluated in combination with SPR741, fixed at either 4 or 8 mg/L, the MIC values were highly variable, ranging from 0.5 to 256 mg/L.
- The MIC values determined using fixed ratios of PIP/TAZ to SPR741 were far less variable than those determined using fixed concentrations of SPR741, with values ranging from 4 to 32 mg/L.

Table 1. Known resistance mechanisms and susceptibility results of the challenge isolate panel evaluated in the one-compartment *in vitro* infection model

Isolate	Known Resistance Mechanisms	MIC Values (mg/L)							
		Piperacillin Alone	PIP/TAZ	SPR741 Alone	PIP/TAZ + SPR741 at 4 mg/L	PIP/TAZ + SPR741 at 8 mg/L	PIP/TAZ + SPR741 2:1 ratio	PIP/TAZ + SPR741 1:1 ratio	PIP/TAZ + SPR741 1:2 ratio
K. pneumoniae AR0010	OmpK35, ogxA, fosACMY-94, SHV-1	>32	64	>32	16	8	16	8	8
E. coli A10085	CMY-2, OmpF	>32	64	32	32	2	16	8	4
K. pneumoniae AR0107	OXA-9, TEM-1A, SHV-83, CTX-M-2, OXA-10, OmpK35, OmpK36	>32	>256	32 >32	256	16, 32	32	16	8
E. coli 12466	CMY-2, CTX-M-10, TEM-1	>32	128	>32	32	8	16	8	8
E. coli 21711	CTX-M-15	32	32	>32	8	2	8	8	4
E. coli 34268	CTX-M-15, OXA-48, TEM-1	>32	256	32, >32	64	8	4	4	4
K. pneumoniae 40031	CTX-M-15, SHV-11	>32	256	32, >32	4	0.5	4	4	4

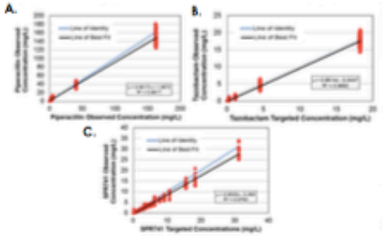
Note: All MIC values shown represent modal values.

RESULTS

Pharmacokinetic Analysis

- As shown in **Figure 2** and evidenced by the r^2 values of greater than 0.95 and the slopes of >0.90 for the relationships between observed and targeted concentrations profiles (which represented less than 10% deviation from identity), concentration-time profiles for piperacillin, tazobactam and SPR741 in the one-compartment *in vitro* infection model were simulated well.

Figure 2. The relationships between observed and targeted piperacillin (A), tazobactam (B), and SPR741 (C) concentrations from a subset of completed *in vitro* studies



Dose-Ranging Studies

- The dose-ranging studies performed provided insight into the inter-isolate variability for PK-PD relationships for SPR741 when administered in combination with a standard PIP/TAZ dosing regimen.
  - SPR741 AUC values of 8.53 to 281 mg•h/L produced results ranging from failure to bacterial stasis in all isolates evaluated.

PK-PD Analyses

- **Figure 3** represents the relationship between change in $\log_{10}$ CFU/mL from baseline at 24 hours and the SPR741 AUC to SPR741 potentiated PIP/TAZ MIC, using MIC values determined using either fixed SPR741 values of 4 and 8 mg/L or at PIP/TAZ:SPR741 ratios of 1:2, 1:1, and 2:1.
- **Figure 4** represents the relationship between change in $\log_{10}$ CFU/mL from baseline at 24 hours and the SPR741 free-drug AUC:MIC ratio, where SPR741 potentiates PIP/TAZ using a fixed ratio of 1:2.
  - As evidenced by the r^2 of 0.688 and dispersion of data across the x-axis, the free-drug AUC:MIC ratio of 1:2 describes the data well.
  - The magnitude of the free-drug AUC:MIC ratio required for net bacterial stasis and a 1-$\log_{10}$ CFU/mL reduction in bacterial burden was 9.66 and 27.0, respectively.

Figure 3. Evaluation of relationships between $\log_{10}$ CFU/mL from baseline at 24 hours and SPR741 free-drug AUC:MIC ratio based on MIC values determined using PIP/TAZ:SPR741 ratios of 1:2, 1:1, and 2:1, as well as PIP/TAZ in combination with fixed SPR741 concentrations of 8 and 4 mg/L

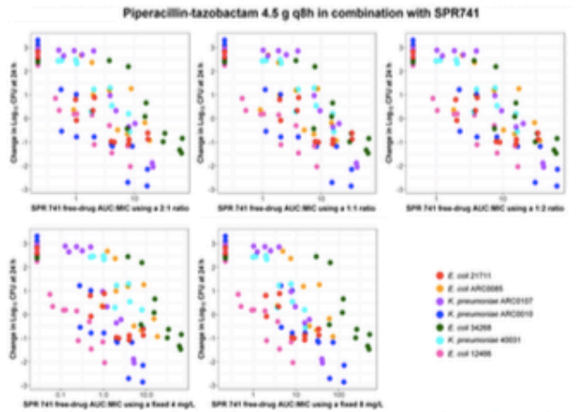
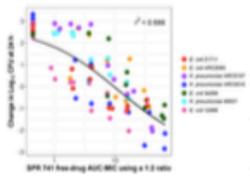


Figure 4. The relationship observed between change in $\log_{10}$ CFU/mL from baseline at 24 hours and SPR741 free-drug AUC:MIC ratio of 1:2, based on co-modeling of data for all clinical isolates



CONCLUSIONS

- Results of MIC assays and dose-ranging studies conducted using the one-compartment *in vitro* model demonstrated that SPR741 enhances the effectiveness of PIP/TAZ against drug-resistant isolates.
- The PK-PD relationship for which SPR741 free-drug AUC:MIC ratio was based on the MIC determined using a fixed 1:2 ratio of PIP/TAZ to SPR741 when given in combination with PIP/TAZ 4.5 g q8h best described the data.
  - SPR741 free-drug AUC:MIC ratios associated with net bacterial stasis and a 1-$\log_{10}$ CFU/mL reduction from baseline at 24 hours were 9.66 and 27.0, respectively.
- Data from these one-compartment *in vitro* infection model studies will be useful to support SPR741 dose selection in combination with PIP/TAZ for future studies.

REFERENCES

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2. Zosyn (piperacillin/tazobactam) package insert. Philadelphia, PA: Pfizer; 2017.

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