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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 Species.

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ABSTRACT

Background: SPR741 is a novel polymyxin B derivative with minimal intrinsic antibacterial activity and reduced nephrotoxicity. SPR741 interacts with the outer membrane of gram-negative (G-) bacteria enhancing penetration of co-administered antimicrobial compounds such as piperacillin-tazobactam (TZP).
Materials/methods: The antibacterial activity of TZP with or without 8 mg/L SPR741 was assessed against 30 isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* species using the CLSI broth microdilution MIC/MBC methods. Selected susceptible, multidrug-resistant, and extended-spectrum beta-lactamase (ESBL) producing isolates of *E. coli* and *K. pneumoniae* were also assayed using a CLSI time-kill method.
Results: SPR741 at 8 mg/L reduced the MICs of 19/22 TZP susceptible isolates by 4-48 fold. SPR741 drove the MICs of 7/8 TZP resistant isolates from ≥64 mg/L to ≤4 mg/L, into the susceptible range using the CLSI breakpoint of 16 mg/L, and the MBC/MIC ratio of 1 was observed for the 7 resistant isolates. TZP in combination with SPR741 was bactericidal at 1-4x MIC against 10/10 *E. coli*, 9/10 *K. pneumoniae*, and 8/10 *Enterobacter* spp. TZP in combination with SPR741 demonstrated a 3log10 CFU/mL reduction in susceptible, ESBL+ and TZP-resistant *E. coli* as well as susceptible and ESBL+ K. pneumoniae at 4x-8x MIC within four to eight hours, similar to TZP alone but at a significantly lower concentration of TZP.
Conclusions: These data illustrate that SPR741 significantly enhances the antimicrobial potency and cidality 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 polymyxin B derivative with minimal intrinsic antibacterial activity and reduced nephrotoxicity. SPR741 interacts with the outer membrane of gram-negative (G-) bacteria, compromising the integrity of the lipopolysaccharide (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 (at 8 mg/L) was assessed *in vitro vs.* 30 clinically relevant susceptible, multidrug-resistant (MDR) and TZP-resistant isolates of *E. coli*, *K. pneumoniae*, and *Enterobacter* species using CLSI method M7-A10 to determine broth microdilution MICs and CLSI method M26A to determine minimal bactericidal concentrations (MBC).
- Selected susceptible, MDR, and extended-spectrum beta-lactamase (ESBL) producing isolates of *E. coli* and *K. pneumoniae* were also assayed using the CLSI time-kill method M26A. In brief, cultures were grown to log phase in CAMHB, diluted to ~2.0E+05 CFU/mL in 10mL CAMHB containing antimicrobials at 2x-32x MIC, and incubated at 35±2°C. Viability was determined by plating for CFU/mL on agar over 24h.

RESULTS

Figure 1. Kill kinetics profiles of SPR741, TZP, MEM, and SPR741/TZP combinations at various concentrations

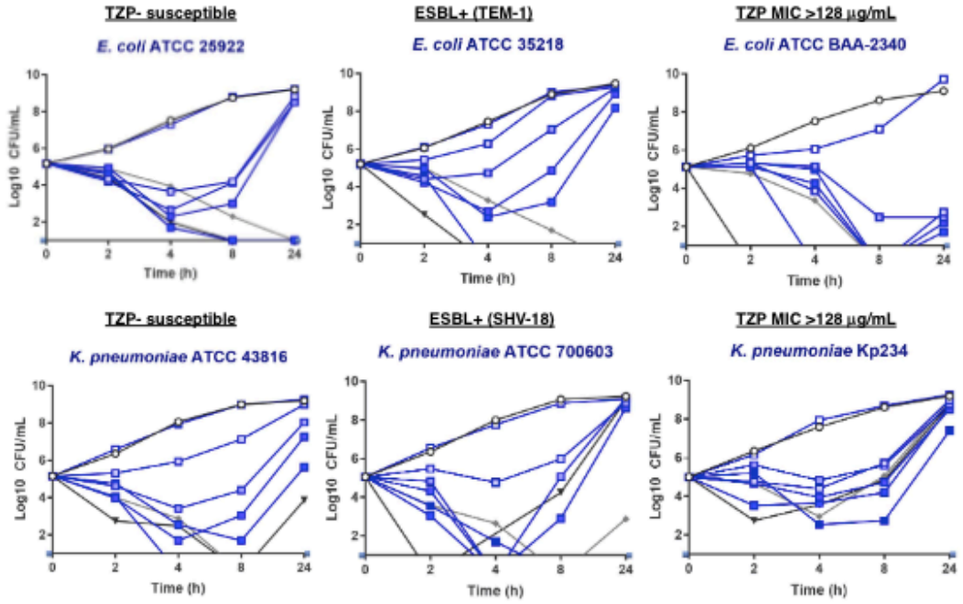
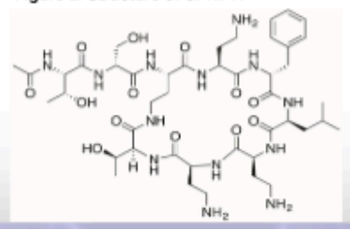


Figure 2. Structure of SPR741



- TZP in combination with SPR741 demonstrated a 3log10 CFU/mL reduction in susceptible, ESBL+ and TZP-resistant *E. coli* as well as susceptible and ESBL+ *K. pneumoniae* at ≥8x MIC within four to eight hours.
- TZP alone at 4x MIC achieved a similar effect within 8 hours against the same strains.
- Results are similar to TZP alone, but at a significantly lower concentration of TZP.

Table 1. MIC / MBC of TZP and SPR741/TZP vs. 30 Isolates

Organism	Isolate ID	TZP MIC (µg/mL) n1	TZP MIC (µg/mL) n2	TZP MBC (µg/mL) n1	TZP MBC (µg/mL) n2	TZP + SPR-741 MIC (µg/mL) n1	TZP + SPR-741 MIC (µg/mL) n2	TZP + SPR-741 MBC (µg/mL) n1	TZP + SPR-741 MBC (µg/mL) n2
E. coli	01CAE05	1	1	2	2	0.25	0.25	0.25	0.25
	01CAE11	2	2	2	2	≤0.125	≤0.125	0.25	≤0.125
	07CAE31	16	16	16	16	0.25	0.5	1	2
	07CAE33	16	16	16	16	0.5	0.5	0.5	0.5
	10CAE06	4	4	4	4	4	8	4	8
	10CAE16	2	2	2	2	0.25	0.5	0.25	0.5
	10CAE22	4	8	4	8	≤0.125	≤0.125	≤0.125	≤0.125
	ATCC 25922	4	4	4	4	≤0.125	≤0.125	≤0.125	≤0.125
	ATCC 35218	1	1	2	1	≤0.125	≤0.125	≤0.125	≤0.125
	ATCC BAA 2340	>128	>128	>128	>128	2	4	2	4
K. pneumoniae	Kp218	64	>128	64	>128	0.25	0.25	0.25	0.25
	Kp224	>128	>128	>128	>128	0.25	0.25	0.25	0.25
	Kp234	>128	>128	>128	>128	0.25	0.25	0.25	0.25
	Kp309	16	16	16	16	1	1	1	1
	Kp310	4	2	4	8	≤0.125	0.25	≤0.125	0.25
	Kp339	>128	>128	>128	>128	128	128	128	128
	Kp420	16	16	16	16	4	4	4	4
	Kp450	4	4	4	4	0.25	0.25	0.25	0.25
	ATCC 43816	4	4	128	32	≤0.125	0.25	>128	>128
	ATCC 700603	16	16	16	16	1	2	1	4
Enterobacter spp.	EN29	>128	>128	>128	>128	1	2	1	2
	EN3635	4	4	4	4	1	1	1	1
	EN8609	8	8	8	8	0.5	2	0.5	2
	EN27	2	2	64	64	0.5	0.5	64	64
	EN28	>128	>128	>128	>128	≤0.125	≤0.125	≤0.125	≤0.125
	EN30	2	2	4	2	0.5	0.5	2	8
	EN31	4	8	4	8	≤0.125	≤0.125	≤0.125	≤0.125
	EN32	16	32	16	128	32	16	32	32
	EN33	>128	>128	>128	>128	1	1	1	1
	EN1636	2	2	32	32	4	4	4	4

- SPR741 reduced the MICs of 19/22 TZP susceptible isolates by 4 - 48 fold using the CLSI breakpoint of 16 mg/L.
- SPR741 reduced the MICs of 7/8 TZP resistant isolates from ≥64 mg/L to ≤4 mg/L, into the susceptible range, with an MBC/MIC ratio of 1 was observed for the 7 resistant isolates.
- TZP in combination with SPR741 was bactericidal at 1-4x MIC against 10/10 *E. coli*, 9/10 *K. pneumoniae*, and 8/10 *Enterobacter* spp.

CONCLUSIONS

These data illustrate that SPR741 significantly enhances the antimicrobial potency and cidality 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

[1]CLSI M07-A10 Methods for Dilution Antimicrobial Susceptibility Testing for Aerobic Bacteria
[2]CLSI M26A Methods for Determining Bactericidal Activity

ACKNOWLEDGMENTS

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