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Bactericidal Activity of Ceftazidime 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 ceftazidime (CAZ).

Materials/methods: The antibacterial activity of CAZ with and without 8 mg/L SPR741 was assessed against clinically relevant susceptible, multidrug-resistant and CAZ-resistant isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter* species using the CLSI broth microdilution MIC/MBC methods. Selected ceftazidime susceptible and resistant isolates of *E. coli* and a ceftazidime resistant isolate of *K. pneumoniae* were also assayed using a CLSI time-kill method.

Results: SPR741 at 8 mg/L reduced MICs of CAZ against 23/30 *E. coli* strains by 4-64 fold, 5/30 by 2-fold; and reduced MICs of CAZ against 10/30 *K. pneumoniae* strains by 4-32 fold, 10/30 by 2-fold. SPR741 drove the MICs of 12/19 CAZ resistant *E. coli* and 4/24 CAZ resistant *K. pneumoniae* isolates into the susceptible range using the CLSI breakpoint of 4 mg/L. CAZ in combination with SPR741 at 8 mg/L was bactericidal at the MIC against 23/30 tested *E. coli*, *K. pneumoniae*, and *Enterobacter* isolates. CAZ in combination with SPR741 demonstrated a 3log10 CFU/mL reduction of CAZ susceptible *E. coli* ATCC 25922 viability at 2x MIC within four hours. CAZ + SPR741 showed a similar rapid bactericidal effect against CAZ resistant clinical isolates *E. coli* 10CAE22 and *K. pneumoniae* Kp450 at 4x and 2x MIC within four hours, respectively, similar to CAZ alone but at a significantly lower concentration of CAZ.

Conclusions: These data illustrate that SPR741 significantly enhances the antimicrobial potency and cidality of CAZ 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 ceftazidime (CAZ) when co-administered.

METHODS

- The potency and bactericidal activity of CAZ alone and in combination with SPR741 (at 8 mg/L) was assessed *in vitro vs.* 30 clinically relevant susceptible, multidrug-resistant (MDR) and CAZ-resistant isolates of *E. coli*, *K. pneumoniae*, and *Enterobacter* species using CLSI method M7-A10[1] to determine broth microdilution MICs and CLSI method M26A[2] to determine minimal bactericidal concentrations (MBC).
- CAZ susceptible and resistant isolates of *E. coli* and a CAZ resistant isolate of *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.

RESULTS

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

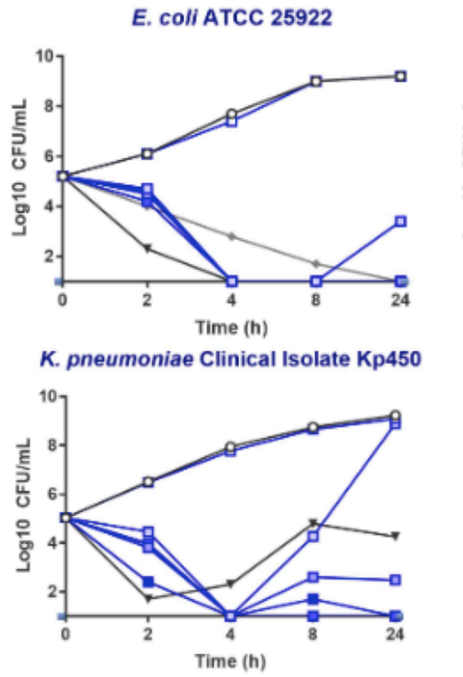
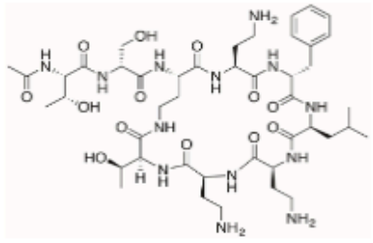


Figure 2. Structure of SPR741



- CAZ in combination with SPR741 demonstrated a 3log$_{10}$ CFU/mL reduction of CAZ susceptible *E. coli* ATCC 25922 viability at 2x MIC within four hours.
- CAZ/SPR741 showed a similar rapid bactericidal effect against CAZ resistant clinical isolates *E. coli* 10CAE22 and *K. pneumoniae* Kp450 at 4x and 2x MIC within four hours, respectively, similar to CAZ alone but at a significantly lower concentration of CAZ.

Table 1. MIC / MBC of CAZ +/- SPR741 at 8 mg/L vs. 30 Isolates

Organism	Isolate ID	MIC / MBC (µg/mL)							
		CAZ		CAZ + SPR-741		Levofloxacin		Meropenem	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	01CAE05	32	32	4	4	1	1	0.06	0.06
	01CAE11	8	8	0.5	0.5	>16	>16	0.06	0.06
	07CAE31	64	64	8	8	16	16	0.06	0.06
	07CAE33	16	64	4	64	16	>16	0.06	0.12
	10CAE06	>64	>64	64	64	0.5	0.5	0.06	0.06
	10CAE16	8	8	2	2	16	16	0.06	0.06
	10CAE22	16	16	2	2	16	16	0.06	0.06
	ATCC 25922	0.25	0.25	≤0.06	0.12	0.03	0.03	0.06	0.06
	ATCC 35218	0.12	0.12	≤0.06	≤0.06	0.03	0.03	0.03	0.03
	ATCC BAA 2340	>64	>64	32	32	>16	>16	4	8
K. pneumoniae	Kp218	>64	>64	32	32	>16	>16	0.25	0.5
	Kp224	>64	>64	64	64	>16	>16	0.25	0.25
	Kp234	>64	>64	>64	>64	>16	>16	0.5	0.5
	Kp309	1	1	0.5	0.5	>16	>16	0.12	0.12
	Kp310	0.25	0.25	≤0.06	≤0.06	0.03	0.03	0.12	0.12
	Kp339	>64	>64	64	64	>16	>16	>16	>16
	Kp420	64	64	64	64	16	16	0.25	0.25
	Kp450	32	32	4	4	>16	>16	0.25	0.25
	ATCC 43816	0.25	2	0.12	4	0.06	0.06	0.25	4
	ATCC 700603	32	32	16	16	0.5	0.5	0.12	0.12
Enterobacter spp.	EN29	>64	>64	32	32	4	4	8	8
	EN3635	2	2	2	2	4	4	0.12	0.12
	EN8609	2	2	0.25	0.25	0.03	0.03	0.06	0.12
	EN27	0.25	0.25	0.25	0.25	0.12	0.12	0.12	0.25
	EN28	64	64	4	4	8	8	0.25	0.25
	EN30	0.12	4	0.12	8	0.06	0.06	0.06	1
	EN31	4	4	0.12	0.12	16	>16	0.06	0.06
	EN32	64	64	32	32	0.06	0.06	0.25	0.25
	EN33	>64	>64	1	1	>16	>16	>16	>16
	EN1636	0.5	0.5	1	1	0.06	0.06	0.12	0.12

- The addition of SPR741 enhanced the potency of CAZ against susceptible isolates and caused the MICs of 8/19 CAZ resistant isolates into the susceptible range using the CLSI breakpoint of 4 mg/L.
- The MBC/MIC ratio of CAZ was 1-4 against 20/22 *E. coli*, *K. pneumoniae*, and *Enterobacter* isolates with MICs ≤64 mg/L.
- CAZ in combination with SPR741 at 8 mg/L was bactericidal at the MIC against 23/29 *E. coli*, *K. pneumoniae*, and *Enterobacter* isolates with MICs ≤64 mg/L.

CONCLUSIONS

These data demonstrate that SPR741 enhances the potency of CAZ and the combination exhibits robust bactericidal activity against clinically relevant species of Enterobacteriaceae, and thus represents a promising treatment option for infections caused by CAZ 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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