Poster #3884

Assessment of the In Vivo Efficacy of SPR741 in Combination with Azithromycin against Multidrug Resistant Enterobacteriaceae Isolates in the Neutropenic Murine Thigh Infection Model

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ABSTRACT

Background: SPR741 is a novel agent with structural similarity to polymyxins that is capable of potentiating the activity of various classes of antibiotics. Previously published studies indicated that although Enterobacteriaceae (EB) isolates had minimal susceptibilities to azithromycin (AZM), the *in vitro* antimicrobial activity of AZM against EB was enhanced when combined with SPR741. The current study evaluated the *in vivo* efficacy of human-simulated regimens (HSR) of AZM and SPR741 alone and in combination against multidrug resistant (MDR) EB.

Methods: We studied 30 MDR EB isolates expressing a wide spectrum of β-lactamases (ESBL, NDM, VIM and KPC) inclusive of a subset of isolates positive for genes conferring macrolide resistance (*mphA, mphE, ermB* and *msr*). The MICs of AZM, SPR741, and the combination of AZM+SPR741 (using a fixed concentration of SPR741= 8 mg/L) were determined in triplicate using broth microdilution. ICR mice were rendered transiently neutropenic, and the thighs were inoculated with bacterial suspensions of 10^7CFU/ml. HSR of AZM and SPR741 equivalent to clinical doses of 500 mg IV q24h and 400 mg q8h IV (1h infusion), respectively, as monotherapies and in combination were developed. Treatment mice were administered the AZM, SPR741 or AZM+SPR741 HSR, while control mice were vehicle-dosed. Efficacy was assessed as the change in $\log_{10}$CFU/thigh at 24 h compared with 0h.

Results: MICs for AZM, SPR741 and AZM+SPR741 were between 8 to >128, 16 to >512, and 0.125 to >32 mg/L and MIC50 was 32, 64 and 1 mg/L, respectively. The average $\log_{10}$CFU/thigh at 0h across all isolates was 5.80 ± 0.30. At 24 h, the bacterial burden increased by an average magnitude of 2.75 ± 0.85 $\log_{10}$ CFU/thigh in the untreated control mice. Treatment with AZM alone was associated with net growth of 2.60 ± 0.83 log10CFU/thigh, SPR741 alone achieved stasis to 1 log kill relative to starting inoculum for 2/30 (6.7%) isolates with an average bacterial burden of 2.02 ± 1.17 $\log_{10}$ CFU/thigh. Among isolates with AZM MIC ≤16 mg/L, treatment with AZM+SPR741was associated with an average reduction in bacterial burden of -0.53 ± 0.82 $\log_{10}$CFU/thigh and stasis to 1-log kill was observed in 9/11 isolates (81.8%). By contrast, isolates with AZM MIC ≥ 32mg/L displayed an average net growth of 1.80 ± 1.41 $\log_{10}$CFU/thigh and stasis to 1-log kill was achieved in 1/19 isolates (5.3%).

Conclusion: Combination therapy of AZM+SPR741 HSR showed promising efficacy against MDR EB for isolates with AZM MIC≤16 mg/L, including those producing a variety of β-lactamases. These data support a potential role for AZM+SPR741 for treatment of infections due to MDR EB.

INTRODUCTION

- SPR741 is a novel cationic peptide with structural similarities to polymyxin B. SPR741 exerts its effect by means of binding interactions with the outer membrane, resulting in disruption of membrane integrity.
- Initial *in vitro* studies demonstrated a potentiation of a multitude of conventional agents, including AZM in the presence of SPR741 against a number of ATTC isolate strains.
- The potential for efficacy of a macrolide/SPR741 combination against MDR clinical Enterobacteriacae could provide a means to circumvent inactivation by β-lactamases commonly expressed by these isolates

OBJECTIVES

- To evaluate the efficacy of HSR of SPR741 and AZM alone and in combination against MDR EB isolates (*Escherichia coli* and *Klebsiella pneumoniae*), including those known to produce KPC, ESBL and MBL and isolates positive for macrolide-resistance genes in the neutropenic murine thigh infection model.

MATERIALS and METHODS

- Thirty MDR clinical Enterobacteriaceae isolates, including 22 CRE, were selected from within the Center for Anti-Infective Research and Development library as well as the FDA-CDC Antimicrobial Resistance Isolate Bank (1).
- Analytical grade standard of SPR741 was utilized for *in vitro* and *in vivo* testing (Lot #151015). Analytical grade standard of AZM was used for *in vitro* testing (Lot #036M4776V, Sigma Aldrich, St. Louis, MO). For *in vivo* studies, commercial vials of AZM were purchased from Cardinal Health (Dublin, OH) (Lot # 6113162, Fresenius Kabi, USA. Lake Zurich IL).
- The MICs of the AZM, SPR741, and the combination of AZM with SPR741 (at fixed concentration of the latter at 8 mg/L) were assessed for these 30 isolates in triplicate (2).
- Pharmacokinetic studies for AZM and SPR741 were carried out to identify regimens that provided exposures similar to those achieved in humans following the intravenous administration of 500 mg q24h and 400mg q8h (1-h infusion) of AZM and SPR741, respectively, when given as monotherapies or in combination (**Tables 1 - 2).**
- Thigh infection with each of the test isolates was produced by intramuscular injection of 0.1 mL of the inoculum into each thigh of the mice 2 hours prior to the initiation of antimicrobial therapy.
- The predetermined regimens were studied in groups (n=3) over a 24-hour treatment period. Control animals received the diluent vehicle in the same volume, route, and schedule as the most frequent dosed drug regimen. treatment mice were administered AZM HSR, SPR741 HSR and the combination AZM /SPR741 HSR.
- Efficacy was quantified by the change in bacterial density ($\Delta\log_{10}$ CFU) obtained in the mice after 24h relative to the 0h untreated controls (**Figure 1 a-c).**

RESULTS

Table 1 . Comparative *f*AUC for SPR741 and AZM HSR

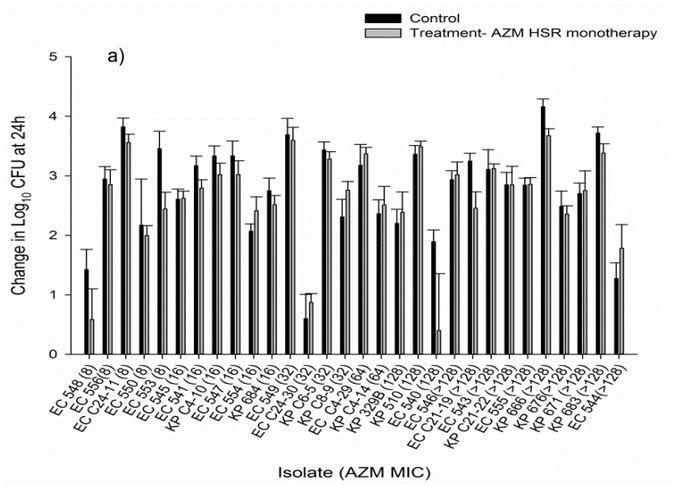
Species	AZM Dose q24h	Regimen	$fAUC_{0\text{-}24}$ (mg•h/L)
Human	500 mg	AZM	8.93
Mouse	10.5 mg/kg	AZM alone	10.56
	10.5 mg/kg	AZM + SPR741 HSR	6.14
	14 mg/kg	AZM + SPR741 HSR	8.97

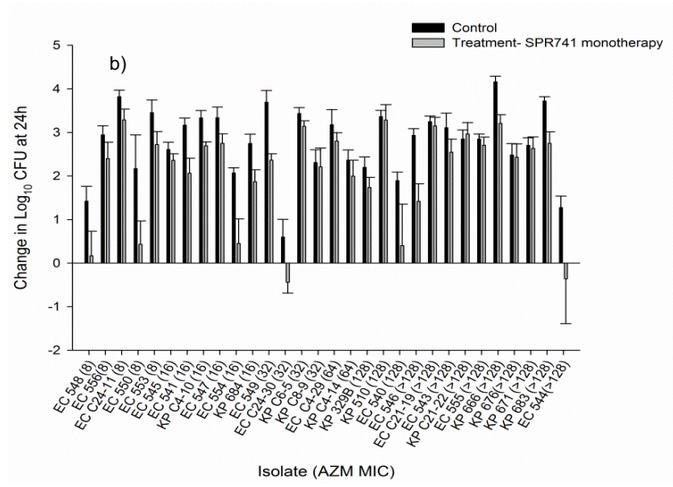
Table 2 . Comparative %*f*T>MIC for SPR741 and AZM HSR

	%*f* T>MIC								
MIC (mg/L)	0.06	0.13	0.25	0.5	1	2	4	8	$fAUC_{0\text{-}24}$ (mg•h/L)
Human[a]	100	100	100	100	85.8	55	24.2	0.8	68.77
Murine HSR[b]	100	100	100	97.5	81.3	55	26.3	5	70.68
a 400 mg q 8h (1h infusion)									
b 0hr 36mg/kg, 1.5hr 33mg/kg, 3.25hr 23mg/kg, 5hr 16m/kg, 6.5hr 12m/kg q 8h									

RESULTS (CONTINUED)

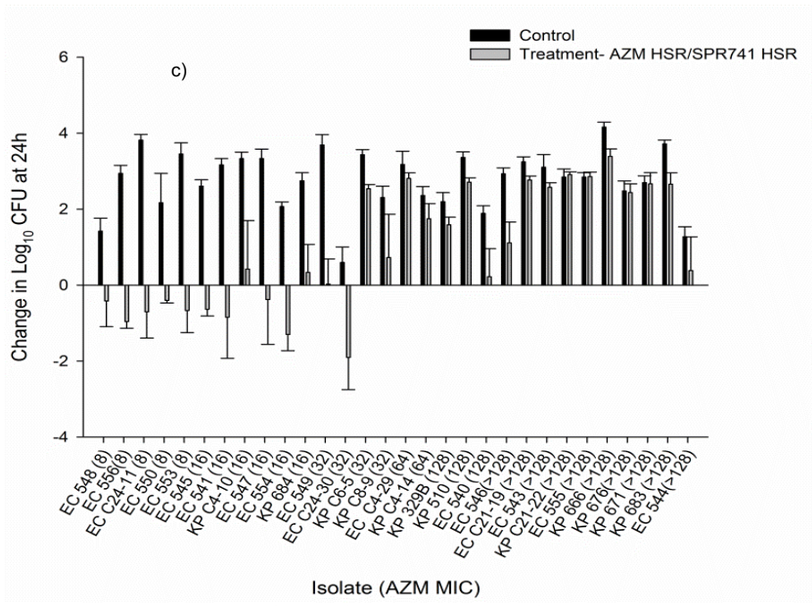
Figures 1. Average change in $\log_{10}$ CFU ± SD at 24 hours with a) humanized AZM, b) SPR741 and c) combination therapy in a neutropenic murine thigh model





Treatment with AZM alone was associated with net growth among all tested isolates.

SPR741 alone achieved stasis to 1 log kill relative to starting inoculum for 2/30 (6.7%) isolates with an average bacterial burden of 2.02 ± 1.17 $\log_{10}$ CFU/thigh.



Among isolates with AZM MIC ≤16 mg/L, treatment with AZM+SPR741was associated with an average reduction in bacterial burden of -0.53 ± 0.82 $\log_{10}$CFU/thigh and stasis to 1-log kill was observed in 9/11 isolates (81.8%).

CONCLUSION

Combination therapy of AZM+SPR741 HSR showed promising efficacy against MDR EB for isolates with AZM MIC≤16 mg/L, including those producing a variety of β-lactamases. These data support a potential role for AZM+SPR741 for treatment of infections due to MDR EB.

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