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In vivo Efficacy of Combinations of Novel Antimicrobial Peptide SPR741 and Rifampicin in Neutropenic Murine Pneumonia Models of Gram-Negative Bacterial Infection

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ABSTRACT

Background: Due to increasing levels of antimicrobial resistance of bacteria recovered from patients with VAP and HAP antimicrobial treatment options are often limited to a very narrow range of drugs or drugs with undesirable PK or tolerability profiles. As a result empirical treatment is often initiated using two or more broad-spectrum antimicrobial agents with subsequent treatment revised when culture data is available. There is a well-recognized shortage of new antimicrobial classes in the development pipeline, possibly leading to a crisis where many infections are untreatable with currently available antibiotics. An alternative approach to increase treatment options is potentiation of the available antimicrobials to increase potency and extend spectrum of activity. In these studies we assessed the efficacy of combinations of a novel antimicrobial cationic peptide (SPR741) with rifampicin in murine models of acute pneumonia.
Material/methods: Male ICR mice were rendered neutropenic using 2 doses of cyclophosphamide on days -4 & -1. Mice were infected by intranasal instillation following ketamine/xylazine anesthesia on day 0 with either *K. pneumoniae* (ATCC 43816) or *Acinetobacter baumannii* (ATCC BAA 747). Treatment was initiated 2h post infection with SPR741 (40 or 80mg/kg/dose) and rifampicin (0.375mg/kg/dose administered at 2, 6, 10, 14, 18 and 22 hours post infection for *A. baumannii.* Treatment was initiated 2h post infection with SPR741 (40 or 60mg/kg/dose) and rifampicin (15 or 20mg/kg/dose administered at 2, 10 and 18 hours post infection for *K. pneumoniae.* Control treatments of polymyxin B or tigecycline were included. Mice were euthanized 26h post infection and the lungs quantitatively cultured.
Results: SPR741 and rifampicin were well tolerated when co-administered and all animals continued to the study end. Both isolates demonstrated robust *in vivo* growth of 0.6-3.5Log10cfu/g lung tissue between pre-treatment and vehicle treated mice. Monotherapy with SPR741 at 40mg/kg/dose had little effect on the burdens (no reduction in burden compared to vehicle *K. pneumoniae* and 0.4 Log10cfu/g (*A. baumannii*) reduction in lung burden. Monotherapy with rifampicin at the highest dose had modest effects on the burdens with a maximum reduction of 2.7 (*K. pneumoniae*) and 2.2 Log10cfu/g (*A. baumannii*) lung burden. In contrast combinations of 40mg/kg/dose SPR741 with rifampicin led to highly significant reductions in burden compared to vehicle (5.4 and 4.8Log10cfu/g for *K. pneumoniae* and *A. baumannii* respectively). When treated with combinations using 60 or 80mg/kg/dose SPR741, did not lead to greater reductions possibly indicating the maximal effect had been achieved. In both model the reduction in burden were to below pre-treatment levels indicating cidal activity.
Conclusions: The combination of SPR741 with rifampicin was highly effective at reducing the lung burden of mice infected with *K. pneumonia* and *A. baumannii*. These studies support continued development of novel antimicrobial cationic peptide for the treatment of multi-drug-resistant Gram-negative infections.

INTRODUCTION

The spread of multi-drug resistant Gram negative bacteria appears unstoppable. In some localities bacteria resistant to all available antibiotics are causing infections, effectively taking us back to the pre-antibiotic era.

The Gram negative bacterial cell membrane acts as a barrier to the entry of many antimicrobial agents rendering the bacteria resistant or at best only weakly susceptible to a potentially useful antimicrobial agent. An attractive approach to addressing the lack of treatment options is potentiation of antimicrobial agents to either increase the spectrum of activity or enhance activity.

In these studies the combination of the cationic peptide SPR741 and rifampicin was assessed in neutropenic murine models of thigh muscle infection due to a range of susceptible and MDR *Enterobacteriaceae* and *Acinetobacter baumannii*. The studies were short duration to enable optimum dosing in terms of PK profile.

METHODS

Immunosuppression: Cyclophosphamide was administered at 150mg/kg IP (D-4) 100mg/kg IP (D-1) to induce neutropenia throughout the infection.

Mouse Strain: ICR male (4-6 mice per group) were used in the studies

Infection: Mice were rendered unconscious using ketamine and xylazine inhaled then 0.04mL of a bacterial suspensions were administered IN (20µL per nostril) and mice held upright for 30 minutes. Strains used were *K. pneumoniae* ATCC 43816 and *Acinetobacter baumannii* ATCC BAA 747

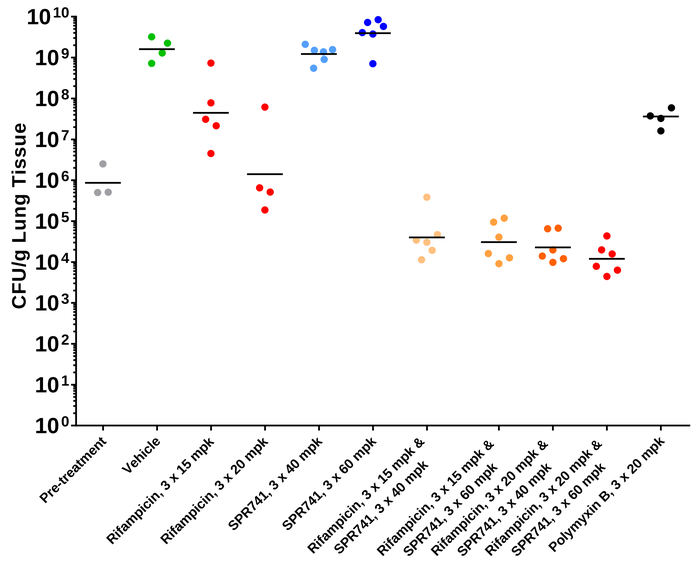
Treatment: S.C. treatment was started 2h post infection. For *K. pneumonia* SPR741 was administered SC at 40 or 60mg/kg/dose and rifampicin at 15 or 20mg/kg/dose at 2, 10 and 18h post infection. For *A. baumannii* SPR741 was administered SC at 40 or 80mg/kg/dose and rifampicin at 0.375mg/kg/dose at 2, 6, 10, 14, 18 and 22h post infection. Polymyxin B and tigecycline control groups were included.

Lungs were harvested at 26h post infection and quantitatively cultured.

RESULTS

Figure 1 and tables 1 and 2. Treatment of *K. pneumonia* ATCC 43816 with SPR741 potentiates the activity of rifampicin in neutropenic murine pneumonia models

Combination treatment following treatment with SPR741 at 40 or 60mg/kg/dose and rifampicin 15 or 20mg/kg/dose or Polymyxin B 20mg/kg (2, 9 and 17h post infection) .



Treatment (mg/kg/dose)	Log_{10} Geometric mean (CFU/g)	Log_{10} change from pre-treatment (CFU/g)
Pre-Treatment	5.94	3.27
Vehicle	9.21	n/a
Rifampicin, 15	7.65	1.56
Rifampicin, 20	6.15	3.06
SPR741, 40	9.09	0.12
SPR741, 60	9.60	-0.39
Rifampicin, 15 + SPR741, 40	4.60	4.60
Rifampicin, 15 + SPR741, 60	4.49	4.72
Rifampicin, 20 + SPR741, 40	4.36	4.85
Rifampicin, 20 + SPR741, 60	4.08	5.13
Polymyxin B 20	7.52	1.69

P-Value Kruskal-Wallis: all pairwise comparisons (Conover-Inman)				
Treatment (mg/kg/dose)	Rifampicin, 15 + SPR741, 40	Rifampicin, 15 + SPR741, 60	Rifampicin, 20 + SPR741, 40	Rifampicin, 20 + SPR741, 60
Vehicle	<0.0001	<0.0001	<0.0001	<0.0001
Rifampicin, 15	<0.0001	<0.0001	<0.0001	<0.0001
Rifampicin, 20	0.0003	0.0001	<0.0001	<0.0001
SPR741, 40	<0.0001	<0.0001	<0.0001	<0.0001
SPR741, 60	<0.0001	<0.0001	<0.0001	<0.0001
Rifampicin, 15 + SPR741, 40		NS	NS	0.0257
Rifampicin, 15 + SPR741, 60			NS	NS
Rifampicin, 20 + SPR741, 40				NS

CONCLUSION

- The combination of SPR741 with Rifampicin was highly effective at reducing the lung burden of mice infected with *K. pneumoniae, or A. baumannii*.
- Efficacy of the combination was achieved using clinically relevant rifampicin treatment regimens
- These studies support continued development of the novel antimicrobial cationic peptide for the treatment of MDR Gram-negative infections.

Figure 2 and tables 3 and 4. Treatment of *A. baumannii* ATCC BAA 747 with SPR741 potentiates the activity of rifampicin in neutropenic murine pneumonia models

Combination treatment following treatment with SPR741 at 40 or 80mg/kg/dose and rifampicin 0.375/kg/dose (2, 6, 10, 14, 18, 22h post infection) or Tigecycline 60mg/kg (2 and 10h post infection) .

Treatment (mg/kg/dose)	Log_{10} Geometric mean (CFU/g)	Log_{10} change from pre-treatment (CFU/g)
Pre-Treatment	7.4	0.4
Vehicle	8.0	N/A
Rifampicin, 0.375	5.8	2.2
SPR741, 40	7.6	0.4
SPR741, 80	3.6	4.5
Rifampicin, 0.375 & SPR741, 40	3.3	4.8
Tigecycline, 60	1.9	6.1

P-Value Kruskal-Wallis: all pairwise comparisons (Conover-Inman)					
Treatment (mg/kg/dose)	Rifampicin, 0.375	SPR741, 40	SPR741, 80	Rifampicin, 0.375 + SPR741, 40	Rifampicin, 0.375 + SPR741, 80
Vehicle	<0.0001	NS	<0.0001	<0.0001	<0.0001
Rifampicin, 0.375		<0.0001	0.0014	0.0002	0.0001
SPR741, 40			<0.0001	<0.0001	<0.0001
SPR741, 80				NS	NS
Rifampicin, 0.375 + SPR741, 40					NS