Monday-561

In Vivo Efficacy of Combinations of Novel Antimicrobial Peptide Spr741 and Rifampicin in Short-Duration Murine Thigh Infection Models of Gram-Negative Bacterial Infection

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ABSTRACT

Objectives: There is an acute shortage of effective antimicrobial agents to treat multi-drug-resistant Gram-negative infection. An attractive approach to addressing the dearth of treatment options is potentiation of antimicrobial agents to either increase the spectrum of activity or enhance activity. In these studies we assessed the efficacy of combinations of a novel antimicrobial cationic peptide (SPR741) with rifampicin (Rif) in murine models of thigh muscle infection.
Methods: Male ICR mice were rendered neutropenic using 2 doses of cyclophosphamide on days -4 & -1. Mice were infected by IM injection into the lateral thigh muscle on day 0 with either *E. coli* (ATCC 25922) *Klebsiella pneumoniae* (IR60 [bla_{NDM-1}]) or ATCC BAA 2146 [bla_{NDM-1}]), *Enterobacter cloacae* (Kp114 [bla_{KPC}] or *Acinetobacter baumannii* (ATCC BAA 747). Treatment was initiated 1h post infection with SPR741 administered at 1, 3.5 and 7h post infection and Rif administered at 1 and 5h post infection). SPR741 was administered at 10, 20 and 40mg/kg/dose, the doses of Rif were based on preliminary dose response experiments (range 0.376-64mg/kg/dose). Mice were euthanized 9h post infection and the thigh muscle quantitatively cultured.
Results: SPR741 and Rif were well tolerated and all animals continued to the study end. All isolates demonstrated robust *in vivo* growth of 1.55-3.4Log_{10}cfu/g thigh tissue between pre-treatment and harvest samples. Monotherapy with SPR741 at 40mg/kg/dose or Rif (at the doses used) had little effect on the burdens and did not achieve stasis against any isolate. In contrast in all models combinations with ≤20mg/kg/dose SPR741 with Rif led to highly significant reductions in burden below stasis (2.2, 3.7, 4.7, 1.6 and 2.9Log_{10}cfu/g below stasis for ATCC 25922, IR60, Kp114, ATCC BAA 2146 and ATCC BAA 747 respectively).
Conclusions: The combination of SPR741 with Rif was highly effective at reducing the thigh burden of mice infected with *E. coli, K. pneumonia, E. cloacae* and *A. baumannii* including strains expressing bla_{KPC} and bla_{NDM-1}. 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

MIC determination: MICs values were determined in cation-adjusted Mueller-Hinton broth, using CLSI guidelines M7-A10 & M100-S26. The MIC of combinations was determined in a checkerboard format based on CLSI M7-A10.

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 26-36g (4 mice per group) in the studies

Infection: Mice were rendered unconscious using inhaled isofluorane anaesthetic then 0.05mL of a bacterial suspensions administered IM to both posterior thigh muscles. Strains used were *E. coli (E.co.)* ATCC 25922, *K. pneumoniae* (*K.p.*) IR60 [bla_{NDM-1}] or ATCC BAA 2146 [bla_{NDM-1}]), *Enterobacter cloacae (E.cl.)* Kp114 [bla_{KPC}] or *Acinetobacter baumannii* (*A.b.*) ATCC BAA 747 0.03mg/kg buprenorphine was administered S.C. whilst mice were still unconscious as pain relief.

Treatment: S.C. treatment was started 1h post infection with 10, 20 or 40mg/kg/dose SPR741 administered SC at 1, 3.5 or 7h post infection. Rifampicin was administered at 1 and 5h post infection IV at 4, 64, 64, 40 and 0.25-20 mg/kg/dose for *E.co.* ATCC 25922, *K.p.* IR60, *K.p.* ATCC BAA 2146, *E.cl.*Kp114 and *A.b.* ATCC BAA 747 respectively. Thighs were harvested at 9h post infection and quantitatively cultured.

RESULTS

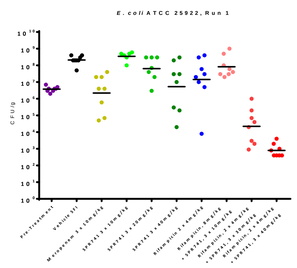
Table 1. The MICs of SPR741 and rifampicin against *E. coli* ATCC 25922, *K. pneumoniae* IR60 and ATCC BAA 2146, *E. cloacae* Kp 114 and *A. baumannii* ATCC BAA 747

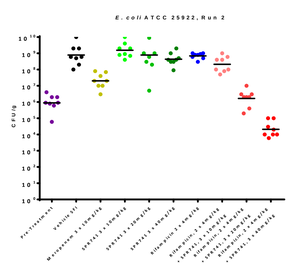
Bacterial strain	MIC SPR741 (µg/mL)	MIC Rifampicin (µg/mL)	MIC Rifampicin (µg/mL) in presence of 8 µg/mL SPR741 *
E. coli ATCC 25922	64	8	≤0.016
K. pneumoniae IR60	128	>128	2
K. pneumoniae ATCC BAA 2146	>128	16	0.25
E. cloacae Kp 114	>128	16	0.125
A. baumannii ATCC BAA 747	16	2	≤0.016

* See poster #SA-502 for more detail

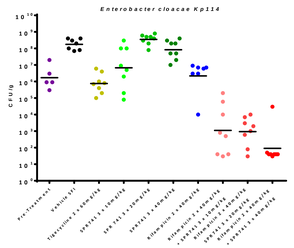
Figures and tables. SPR741 potentiates the activity of rifampicin in short duration neutropenic murine thigh burden models

Combination treatment outcomes following three doses of SPR741 at 10, 20 and 40mg/kg/dose (1, 3.5 and 7h post infection) and 2 doses of rifampicin 0.25-64mg/kg/dose (1 and 5h post infection).





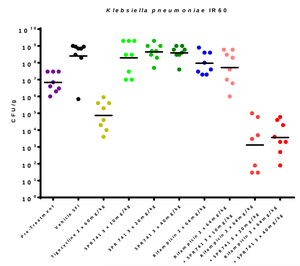
Treatment (mg/kg/dose)	*E. coli* ATCC 25922 Run 1 + 2	
	Log_{10} Geometric mean (CFU/g)	Log_{10} change from pre-treatment (CFU/g)
Pre-Treatment	6.34	0.00
Vehicle SFI	8.69	2.35
SPR741 3 x 40mg/kg	8.09	1.74
Rifampicin 2 x 4mg/kg	8.26	1.92
Rifampicin, 2 x 4mg/kg + SPR741, 3 x 40mg/kg	3.39	-2.95



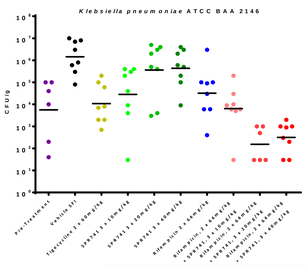
Treatment (mg/kg/dose)	*Enterobacter cloacae* Kp 114	
	Log_{10} Geometric mean (CFU/g)	Log_{10} change from pre-treatment (CFU/g)
Pre-Treatment	6.25	0.00
Vehicle SFI	8.24	1.99
SPR741 3 x 20mg/kg	7.90	1.65
Rifampicin 2 x 40mg/kg	6.36	0.11
Rifampicin 2 x 40mg/kg + SPR741 3 x 10mg/kg	3.06	-3.19

CONCLUSION

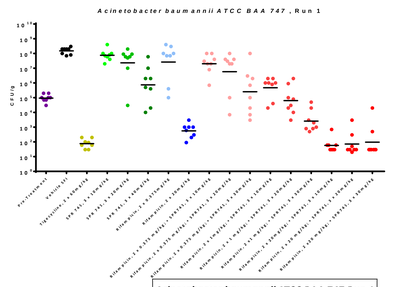
- The combination of SPR741 with Rifampicin was highly effective at reducing the thigh burden of mice infected with *E. coli, K. pneumoniae, E. cloacae* and *A. baumannii* including strains expressing bla_{KPC} and bla_{NDM-1} in a dose dependent manner
- Efficacy of the combination was achieved using clinically relevant rifampicin treatment regimens
- These studies support continued development of the novel antimicrobial cationic peptide for the treatment of MDR Gram-negative infections.



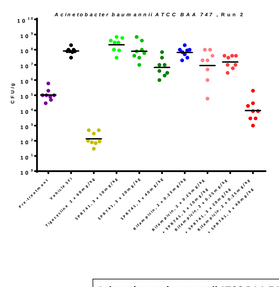
Treatment (mg/kg/dose)	*Klebsiella pneumoniae* IR60	
	Log_{10} Geometric mean (CFU/g)	Log_{10} change from pre-treatment (CFU/g)
Pre-Treatment	6.86	0.00
Vehicle SFI	8.41	1.55
SPR741 3 x 40mg/kg	8.63	1.77
Rifampicin 2 x 64mg/kg	7.96	1.1
Rifampicin 2 x 64mg/kg + SPR741 3 x 20mg/kg	3.13	-3.73
Rifampicin 2 x 64mg/kg + SPR741 3 x 40mg/kg	3.57	-3.29



Treatment (mg/kg/dose)	*Klebsiella pneumoniae* ATCC BAA 2146	
	Log_{10} Geometric mean (CFU/g)	Log_{10} change from pre-treatment (CFU/g)
Pre-Treatment	3.76	0.00
Vehicle SFI	6.16	2.40
SPR741 3 x 40mg/kg	4.52	0.76
Rifampicin 2 x 64mg/kg	4.44	0.68
Rifampicin, 2 x 64mg/kg + SPR741, 3 x 20mg/kg	2.21	-1.55
Rifampicin, 2 x 64mg/kg + SPR741, 3 x 40mg/kg	2.52	-1.24



Treatment (mg/kg/dose)	*Acinetobacter baumannii* ATCC BAA 747 Run 1	
	Log_{10} Geometric mean (CFU/g)	Log_{10} change from pre-treatment (CFU/g)
Pre-Treatment	4.97	0.00
Vehicle SFI	8.18	3.21
SPR741, 3 x 40mg/kg	5.87	0.90
Rifampicin, 2 x 0.375mg/kg	7.42	2.45
Rifampicin, 2 x 1mg/kg + SPR741, 3 x 40mg/kg	3.41	-1.56



Treatment (mg/kg/dose)	*Acinetobacter baumannii* ATCC BAA 747 Run 2	
	Log_{10} Geometric mean (CFU/g)	Log_{10} change from pre-treatment (CFU/g)
Pre-treatment	5.02	0.00
Vehicle SFI	7.92	2.90
SPR741, 3 x 40mg/kg	6.84	1.82
Rifampicin, 2 x 0.25mg/kg	7.82	2.80
Rifampicin, 2 x 0.25mg/kg + SPR741, 3 x 40mg/kg	3.99	-1.03