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In Vivo Efficacy of Tebipenem-Pivoxil (SPR994) in Neutropenic Murine Lung Models of Gram-Negative Bacterial Infection

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ABSTRACT

Background: SPR994 is tebipenem-pivoxil, an orally available carbapenem with broad-spectrum activity against extended-spectrum β-lactamase (ESBL) producing Gram-negative bacteria, currently under development for the treatment of complicated urinary tract infections (cUTI). Herein, we assessed the efficacy of SPR994 in neutropenic murine models of lung infection by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Material/methods: MIC assays were performed with SPR859, the microbiologically active form of SPR994, using CLSI methodology. Male ICR mice were rendered neutropenic using cyclophosphamide on days -4 & -1. Mice were infected intranasally with either *K. pneumoniae* ATCC 43816 (~2.5×10^5 CFU), or *P. aeruginosa* ATCC 27853 (4×10^4 CFU). Treatment initiated 2 h post infection. For *K. pneumoniae* models, SPR994 was administered either as a single oral dose (PO) of 3, 10, or 30 mg/kg, or as three doses of 3.33, 10, 20, or 33.3 mg/kg/dose PO given q8h. For the *P. aeruginosa* model, SPR994 was administered as two doses of 10, 30, 100, or 300 mg/kg/dose PO at 2 and 10 h post infection. Mice were euthanized 26 h (*K. pneumoniae*) or 16 h (*P. aeruginosa*) post infection, and lung tissue quantitatively cultured.

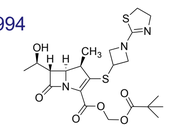
Results: The SPR859 MIC values were 0.06 and 4 mg/L against ATCC 43816 and ATCC 27853, respectively. Both organisms established robust infections resulting in increases in bacterial burden of >3$\log_{10}$ CFU/g over the duration of the studies. Using *K. pneumoniae* ATCC 43816 as the infecting organism, SPR994 reduced the bacterial lung burden relative to vehicle-treated controls following doses of 10 mg/kg/day or greater. At 100 mg/kg/day (administered q8h) SPR994 decreased bacterial lung burden by 2.0 $\log_{10}$ CFU/g relative to pre-treatment. Against *P. aeruginosa* ATCC 27853, 30 mg/kg/dose SPR994 administered q8h resulted in a reduction of 1.3$\log_{10}$ CFU/g relative to pre-treatment. Maximum reductions of 2.4 to 2.6$\log_{10}$ CFU/g below pre-treatment were achieved with 100 and 300 mg/kg/dose SPR994.

Conclusions: These experiments demonstrated that SPR994 was effective at reducing bacterial burden in the lungs of mice in a dose-dependent fashion *in vivo*. Based on these results, further studies to investigate the effectiveness of SPR994 for pneumonia caused by Gram-negative bacteria, including *P. aeruginosa*, are warranted.

INTRODUCTION

SPR994 is tebipenem-pivoxil, an orally available carbapenem with broad-spectrum activity against extended-spectrum β-lactamase (ESBL) producing Gram-negative bacteria, currently under development for the treatment of complicated urinary tract infections (cUTI). Herein, we assessed the efficacy of SPR994 in neutropenic murine models of lung infection by *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Figure 1. Structure of SPR994



METHODS

Antimicrobial susceptibility testing was performed with SPR859, the microbiologically active form of SPR994, using CLSI methodology. Animal experiments were performed under UK Home Office Licence P2BC7D240, with local ethical committee clearance. Male ICR mice were rendered neutropenic using cyclophosphamide on days -4 & -1 (200 and 150 mg/kg, respectively). Mice were infected intranasally with either *K. pneumoniae* ATCC 43816 (broadly antibiotic-susceptible, ~2.5 × 10^5 CFU), or *P. aeruginosa* ATCC 27853 (inducible AmpC β-lactamase, 4 × 10^4 CFU). Treatment initiated 2 h post infection. For *K. pneumoniae* models (26 h duration), SPR994 was administered either as a single oral dose (PO) of 3, 10, or 30 mg/kg, or as three doses of 3.33, 10, 20, or 33.3 mg/kg/dose PO given q8h. For the *P. aeruginosa* model (15 h duration), SPR994 was administered as two doses of 10, 30, 100, or 300 mg/kg/dose PO at 2 and 10 h post infection. Lung tissue was quantitatively cultured, serially diluted, plated on appropriate media and colony forming units quantified following overnight incubation.

RESULTS

Studies 1 and 2: *K. pneumoniae* ATCC 43816 burden in lung tissue following administration of SPR994 (PO) compared to tigecycline (SC) and/or meropenem (IV) in the neutropenic murine lung infection model. Treatment with SPR994 caused a dose-dependent reduction in lung burden compared to vehicle-treated animals whether administered q24h or q8h PO. When administered q24h, reduction of the lung burden to pre-treatment levels was achieved following administration of 30 mg/kg SPR994. When administered q8h, SPR994 doses greater than 10 mg/kg/dose reduced the bacterial burden to below pre-treatment levels.

Table 1. MIC of test articles used in Studies 1 and 2.

Bacterium	MIC (μg/mL)		
	SPR859 (Tebipenem)	Meropenem	Tigecycline
K. pneumoniae ATCC 43816	0.06	0.03	0.5

Table 2. Study 1 design and terminal lung burden of *K. pneumoniae* ATCC 43816. TIG, tigecycline.

Study Group	Dose (mg/kg/dose)	Regimen (h post-infection)	Geometric Mean Bacterial Burden ($\log_{10}$ CFU/g)	Log Δ *vs* 2 h Control ($\log_{10}$ CFU/g)
2 h	-	-	6.24	-
26 h	-	-	9.24	+2.99
TIG	40	2, 10, 18	5.77	-0.47
SPR994	3	2	9.06	+2.81
	10	2	7.79	+1.55
	30	2	6.25	+0.01

Figure 2. Study 1 scatter plot of geometric mean bacterial burden (CFU/g) 26 hours following infection with *K. pneumoniae* ATCC 43816 and treatment with test articles.

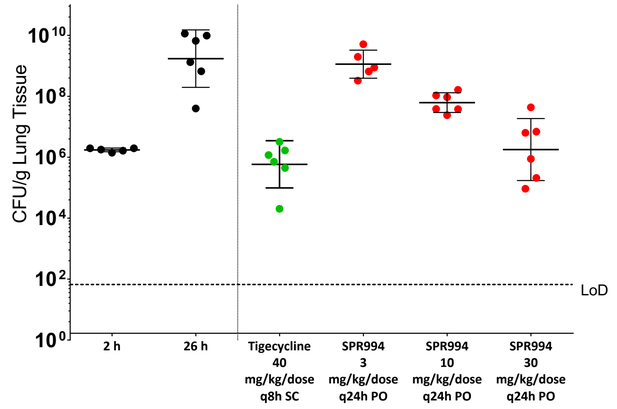
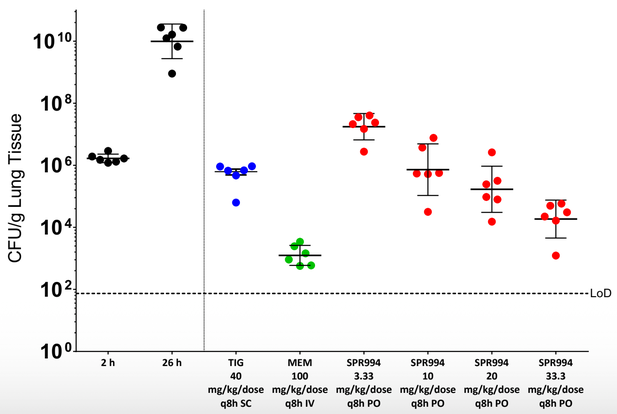


Table 3. Study 2 design and terminal lung burden of *K. pneumoniae* ATCC 43816. TIG, tigecycline; MEM, meropenem.

Study Group	Dose (mg/kg/dose)	Regimen (h post-infection)	Geometric Mean Bacterial Burden ($\log_{10}$ CFU/g)	Log Δ *vs* 2 h Control ($\log_{10}$ CFU/g)
2 h	-	-	6.22	-
26 h	-	-	9.99	+3.77
TIG	40	2, 10, 18	5.68	-0.55
MEM	100	2, 10, 18	3.10	-3.13
SPR994	3.33	2, 10, 18	7.25	+2.75
	10	2, 10, 18	5.86	-0.36
	20	2, 10, 18	5.23	-0.99
	33.3	2, 10, 18	4.27	-1.96

Figure 3. Study 2 scatter plot of geometric mean bacterial burden (CFU/g) 26 hours following infection with *K. pneumoniae* ATCC 43816 and treatment with test articles.



Study 3: *P. aeruginosa* ATCC 27853 burden in lung tissue following administration of SPR994 (PO), polymyxin B (SC) and meropenem (IV). The burden of *P. aeruginosa* ATCC 27853 in the lung was reduced below pre-treatment following 30 mg/kg/dose SPR994 or greater q8h, with a maximum reduction of 2.6$\log_{10}$ CFU/g achieved following of 300 mg/kg q8h.

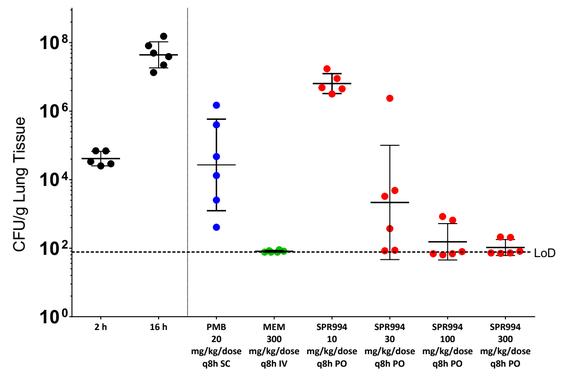
Table 4. MIC of test articles used in Study 3.

Bacterium	MIC (μg/mL)		
	SPR859	Meropenem	Polymyxin B
P. aeruginosa ATCC 27853	4	0.5	1

Table 5. Study 3 design and terminal lung burden of *P. aeruginosa* ATCC 27853. MEM, meropenem; PMB, polymyxin B

Study Group	Dose (mg/kg/dose)	Regimen (h post-infection)	Geometric Mean Bacterial Burden ($\log_{10}$ CFU/g)	Log Δ *vs* 2 h Control ($\log_{10}$ CFU/g)
2 h	-	-	4.61	-
15 h	-	-	7.64	+3.03
MEM	300	2, 10	1.91	-2.71
PMB	20	2, 10	4.43	-0.18
SPR994	10	2, 10	6.80	+2.19
	30	2, 10	3.33	-1.28
	100	2, 10	2.19	-2.43
	300	2, 10	2.02	-2.59

Figure 4. Study 3 scatter plot of geometric mean bacterial burden (CFU/g) 15 hours following infection with *P. aeruginosa* ATCC 27853 and treatment with test articles.



CONCLUSIONS

- Orally-administered SPR994 was effective at reducing the burden of *K. pneumoniae* ATCC 43816 and *P. aeruginosa* ATCC 27853 in the lungs of mice in a dose-dependent fashion in vivo
- These results warrant further investigation into the effectiveness of SPR994 for the treatment of Gram negative bacterial pneumonia