Presentation
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Efficacy of BAL30072 in Murine Lung Infection Models of Multi-Resistant Gram-Negative Bacteria.

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Abstract

Objectives: It is widely acknowledged that new antibiotics active against multidrug-resistant (MDR) Enterobacteriaceae, *P. aeruginosa*, and *A. baumannii* are urgently needed. BAL30072 (SFM) is a novel monosulfactam antibiotic with potent activity against resistant isolates, including those harbouring AmpC β-lactamases, metallo- (class B) or OXA- (class D) beta-lactamases, but less active against strains with high-level expression of class A and D ESBLs and is currently in clinical development. In these studies, we used a neutropenic murine lung burden model to study the efficacy of SFM against a range of MDR Gram-negative bacteria.

Methods: Male ICR mice were rendered neutropenic with 2 doses of cyclophosphamide then infected by intranasal instillation with 2 x *K. pneumoniae,* 1 x *P. aeruginosa* or 1 x *A. baumannii* including isolates expressing bla_{KPC} and bla_{CTX-M}. In PK studies mice were immunosuppressed and infected; blood and BAL samples were collected 10 minutes to 8 hours post infection following a single dose of SFM, levels in ELF were corrected using the plasma:BAL urea levels. In the efficacy studies, treatment started 1h post-infection and administered in the range 3.125 to 400mg/kg/dose IV every two hours equivalent to 12.5 to 1600mg/kg/total compound administered (different dose ranges were used for different strains). Mice were euthanized at 9h post-infection and lung burdens quantified. Dose response curves were used to determine the E_{max} and the ED_{50}.

Results: The PK of SFM in ELF was relatively linear across the dose range assessed (12.5-800mg/kg) following single dosing. SFM entered rapidly and extensively the lungs after IV administration with ELF C_{max} and AUC values approximately 60% of that unbound determined in plasma. Untreated mice demonstrated 1-1.6 log_{10} cfu/g increase in burden. SFM was highly effective against all isolates tested reducing the lung burdens compared to pre-treated burdens by >2.5 log_{10} cfu/g for three strains and ~ 0.8 log_{10} cfu/g for a *K. pneumoniae* expressing bla_{KPC}. For 3 of 4 strains stasis was achieved when *f*T>MIC was over 60%. The ED_{50}s ranged from 18-202mg/kg/dose but were not predicted by the MIC in all cases.

Organism	Resistance type	SFM MIC (µg/mL)	Lung					
			Log_{10} cfu/g pre-treatment	Log_{10} cfu/g vehicle	Log_{10} cfu/g increase	E_{max} Log_{10}R	ED_{50} (mg/kg/dose)	ED_{50} (mg/kg total)
K. pneumoniae	bla_{KPC}	8.5	8.20	9.25	1.05	0.79	133	532
K. pneumoniae	bla_{CTX-M}	5.86	7.31	8.63	1.32	2.66	18	72
P. aeruginosa	Inducible *ampC*	1.9	6.49	7.73	1.25	2.63	79	316
A. baumannii	None	0.25	7.99	8.96	0.97	2.72	202	808

Conclusions: SFM is highly active against MDR bacteria including those expressing bla_{CTX-M} and bla_{KPC}. Treatment of all isolates led to large reductions in lung burden compared to vehicle control treatment. These data suggest SFM could be an effective treatment option for MDR and carbapenemase-producing Gram-negative bacteria and support further clinical studies.

Introduction

There is an urgent need for new antimicrobial agents to treat multi-drug resistant Gram-negative bacteria. The last 20 years have seen seen a rapid increase in resistance to beta-lactams, which has reduced the available treatment options and in some cases led to infections where there are no effective antibacterial agents available.

BAL30072 (SFM) is a siderophore-containing monosulfactam currently in Phase I of clinical development (Basilea Pharmaceutica International Limited). It is active against multi-resistant Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp., including strains producing Ambler Class A, B and D carbapenemases [1-4].

This study examined the *in vivo* activities of BAL30072 in neutropenic murine pneumonia models against a range of bacterial strains in an attempt to understand the correlation between drug concentrations and efficacy.

Methods

Immunosuppression: Cyclophosphamide 200mg/kg IP (Day-4) & 150mg/kg IP (Day-1).

Mouse Strain: ICR male mice (7 per group).

MICs: CLSI M07A9 (repeated >20 times/isolate).

Infection: Mice anaesthetised with Ketamine/Xylazine IP (100mg/kg Ketamine/6mg/kg Xylazine). Anaesthetised mice were infected with 0.04mL inoculum by intranasal instillation

PK study: BAL30072 (3.125 - 800mg/kg/dose), delivered at 10mL/kg IV. Treatment was initiated 1h post infection and administered once. Plasma and bronchoalveolar lavage (BAL) were collected 15mins-4h post dose. Endothelial lining fluid (ELF) concentrations were corrected using the BAL:Plasma urea ratio

Efficacy studies: BAL30072 (3.125 - 800mg/kg) IV. Treatment was initiated 1h post infection and administered q2h (total of 4 doses). Mice were euthanized 9h post infection.

Mathematical modelling: Results analysed using the sigmoid dose-effect model derived from the Hill equation with E_{max}, ED_{50}, calculated using nonlinear least-squares regression. Correlation between efficacy and PK/PD indices was determined by nonlinear regression.

Results

MIC

MICs of BAL30072 for strains of *K. pneumoniae* ATCC BAA 1705 (bla_{KPC-2}), *K. pneumoniae* NCTC 13465 (bla_{CTX-M}), *P. aeruginosa ATCC 27853 (ampC)and A. baumannii* ATCC BAA 747 were, 8.5, 5.86, 1.93 and 0.25µg/mL respectively and were highly reproducible.

Pharmacokinetics: BAL30072 was well tolerated. The PK of BAL30072 in ELF showed mouse-to-mouse variation but at doses of 200-800mg/kg/dose $T_{1/2}$ in BAL ranged from 40 to 61 minutes (Fig 3).

Efficacy: Untreated mice demonstrated 0.97 to 1.56$_{log10}$ cfu/g increase in burden over 9h.

BAL30072 was highly effective against all isolates with MICs ≤8.5µg/mL. Treatment of three strains with BAL30072 reduced the lung burdens below stasis at optimized doses. In contrast treatment of *K. pneumoniae* ATCC BAA 1705 with BAL30072 at doses of up to 400mg/kg/dose did not achieve stasis (Fig 1).

For 3 of 4 strains stasis was achieved when *f*T>MIC was over 60%. The ED_{50}s ranged from 18-202mg/kg/dose but were not predicted by the MIC in all cases. (Fig 2).

Figure 1. Dose response curves for lung burdens at 9h post-infection with *K. pneumoniae, P. aeruginosa* and *A. baumannii.* Drugs were administered 4 times at q2h and burdens measured 9h post infection.

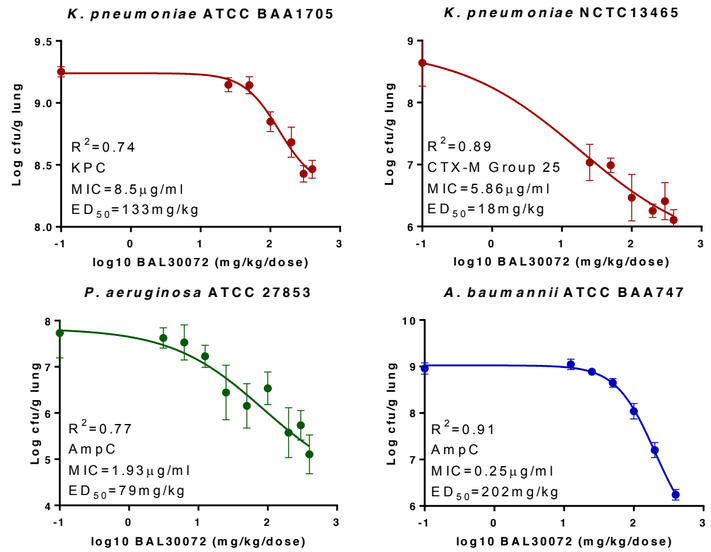

Figure 2. Pharmacodynamic regression lung infected mice treated with BAL30072. The dose data is expressed as the free drug T> MIC. The line drawn through the data points is the best fit line based upon the sigmoid E_{max} formula. The horizontal dotted line represents the burden of organisms in the lungs of mice at the start of therapy.

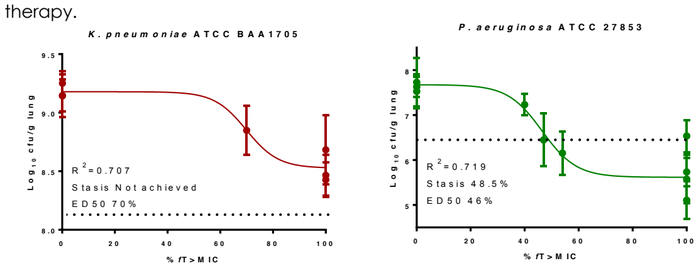

Figure 3. Endothelial lining fluid PK parameters of BAL30072

	12.5mg/kg	50mg/kg	200mg/kg	400mg/kg	800mg/kg
Dosage mg/kg	12.5	50.0	200.0	400.0	800.0
Elimination Half-life (minutes)	n/a	110	53	61	40
Initial concentration µg/mL (15 min)	4.9	7.6	92.5	101.1	310.9
AUC (T0-240) µg-min/mL	384	469	7286	4808	22626

Conclusions

- SFM is highly active *in vitro* and *in vivo* against MDR bacteria including those expressing bla_{CTX-M} and bla_{KPC}.
- AUCs in ELF were approximately 50% of those determined in plasma.
- Treatment of infected mice led to reductions in lung burden for all isolates compared to vehicle control treatment. Treatment of 3 or 4 strains resulted in reductions of burden of >2.5Log_{10}cfu/g lung.
- SFM was effective *in vivo* against isolates with MIC values up to 8.5µg/mL.
- The *in vivo* efficacy of SFM against *K. pneumoniae* and *P. aeruginosa isolates* was time dependent with ED_{50} achieved when *f*T>MIC was >46% for these species.
- These data suggest SFM could be an effective treatment option for MDR and carbapenemase-producing Gram-negative bacteria causing pneumonia

References

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