Presentation
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Efficacy of BAL30072 in Combination with Meropenem in Murine Thigh Infection Models of Multi-Resistant Gram-Negative Bacteria.

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Abstract

Objectives: New antibiotics administered as monotherapy or in combinations that are active against multidrug-resistant Enterobacteriaceae, *P. aeruginosa*, and *A. baumannii* are urgently needed. BAL30072 (SFM) is a novel monosulfactam antibiotic with potent activity against MDR isolates and is currently in phase 1 clinical development in combination with meropenem (MER). In these studies, a neutropenic murine thigh burden model was used to study efficacy of the compounds alone and combinations of SFM and MER against a range of multi-resistant Gram-negative bacteria.
Methods: Male ICR mice were rendered neutropenic with 2 doses of cyclophosphamide then infected IM into both thighs with *E. coli* (resistant (EUCAST) to MER, susceptible to SFM), *K. pneumoniae* (resistant *in vitro* to MER and high MIC to SFM) or *P. aeruginosa* (susceptible to MER *in vitro* but a high MIC to SFM). Treatment was administered 1, 3, 5 and 7 h post infection intravenously using a range of MER, SFM or combination doses (combinations at fixed 1:1, 2:1 or 4:1 MER:SFM ratios). Mice were euthanized at 9 h post-infection and thigh burdens quantified.
Results: The combination of MER and SFM was well tolerated at all doses used. Untreated mice demonstrated 1.4-2.2 log10cfu/g increase in burden. The monotherapy dose response studies confirmed the susceptibility status of the bacterial strains against MER and SFM. Administration of MER and SFM in combination increased the treatment efficacy window against all strains. The largest improvements in efficacy were observed where the isolate was resistant to one of the two monotherapies. Combination was superior in terms of the dose required for (a) 1 log10 reduction compared to vehicle, (b) stasis and (c) 1 log10 reduction compared to pre-treatment burden (a and b only for *K. pneumoniae*). Synergy of the combinations for these tissues burden endpoints was demonstrated as defined by the Lowe Additivity Interaction index with all values <1.0.

		ED_{50} (mg/kg total dose)		
		K. pneumoniae	*P. aeruginosa*	*E. coli*
MER monotherapy		819	297	813
SFM monotherapy		608*	328*	323
MER dose	MER:SFM 1:1	377	111	119
	MER:SFM 2:1	370	137	195
	MER:SFM 4:1	468	144	376
SFM dose	MER:SFM 1:1	377	111	119
	MER:SFM 2:1	185	69	97
	MER:SFM 4:1	117	35	94

*Estimated as E_{max} was not achieved

Conclusions: The combination of meropenem and SFM is highly active against MDR bacteria *in vivo*. For all strains a synergistic effect of the combination could be demonstrated. For all organisms, a combination of meropenem and SFM at a 1:1 ratio led to an impressive increase in efficacy.

Introduction

Effective antimicrobial therapy against bacterial pathogens is becoming increasingly difficult due to the emergence and spread of resistance. Nosocomial spread of MDR Gram-negative species including cephalosporin-resistant Enterobacteriaceae, and *Acinetobacter* producing OXA-type carbapenemases, and MDR *Pseudomonas* limit therapeutic options.

BAL30072 (SFM) is a siderophore-containing monocyclic β-lactam antibiotic 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 and *in vitro* demonstrated synergy with meropenem [1-3] and potent *in vivo* activity [4].

This study examined the *in vivo* activities of SFM and meropenem (MER) combinations in neutropenic murine thigh burden models against a range of bacterial strains to confirm *in vivo* synergy and to define the optimum ratio of SFM and MER.

Methods

Immunosuppression: Cyclophosphamide 150mg/kg IP (Day-4) & 100mg/kg IP (Day-1).

Mouse Strain: ICR male mice (5 per group).

MICs: Performed in accordance with CLSI M07A9

Infection: 0.05mL challenge per mouse by intramuscular injection (IM) of a validated stock into both posterior thigh muscles under inhaled anaesthesia.

Dose ranging studies: Dose response studies were performed with meropenem and BAL30072 delivered as monotherapy at 10mL/kg IV. Treatment was initiated 1h post infection and administered q2h (total of 4 doses). Mice were euthanized 9h post infection.

Methods (continued)

Combination studies: Meropenem and BAL30072 were administered as monotherapy and at fixed ratios of (MER:SFM) 1:1, 2:1 and 4:1 with doses selected to cover the steep part of the dose response. Treatment was initiated 1h post infection and administered q2h (total of 4 doses). Mice were euthanized 9h post infection.

Mathematical modelling: Results were analysed using the sigmoid dose-effect model from the Hill equation with E_{max}, ED_{50}, calculated using nonlinear least-squares regression. Curves were also generated for fT>MIC. Data was plotted in terms of the MER and the BAL30072 dose. Loewe Additivity Interaction Index was calculated for1:1, 2:1 and 4:1 combinations. Values of <1=synergy; 1=additivity; >1=antagonism.

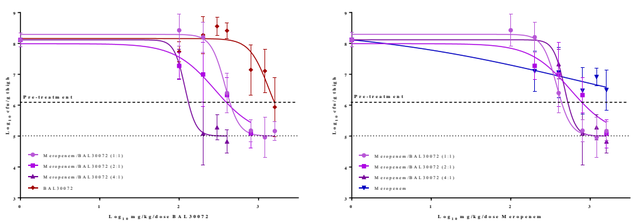
Results

Table 1: In vitro susceptibilities of strains used in efficacy studies

Organism	Strain	Resistance Genotype	BAL30072 MIC (µg/mL)	Meropenem MIC (µg/mL)
K. pneumoniae	ATCC BAA 1705	bla_{KPC-2}	4	>32
P. aeruginosa	ATCC 27853	*ampC*	2	0.5
E. coli	IR3E	bla_{NDM-1}	0.5	>32

Tolerability: Meropenem and BAL30072 were well tolerated at the doses administered

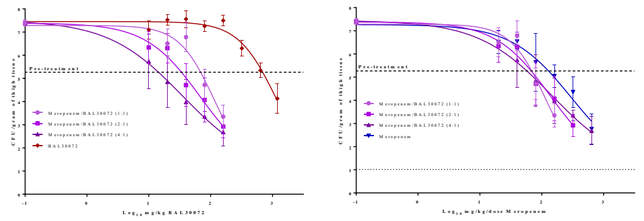
Figure 1. Dose response curves and Loewe Additivity Interaction Index for thigh burdens at 9h post-infection with *K. pneumoniae* ATCC BAA 1705. Drugs were administered 4 times at q2h and burdens measured 9h post infection.



Loewe additivity interaction index

Effect on Burden	Combination 1:1	Combination 2:1	Combination 4:1
1.0 log_{10} below vehicle	1.75	1.63	2.6
Stasis	0.58	0.58	0.48
1.0 log_{10} below stasis	0.69	0.69	0.76

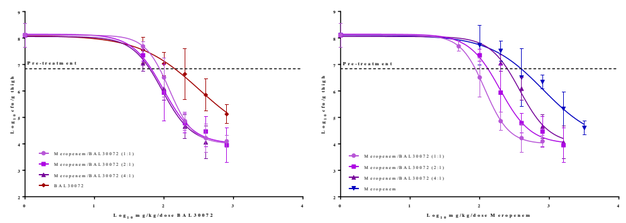
Figure 2. Dose response curves and Loewe Additivity Interaction Index for thigh burdens at 9h post-infection with *P. aeruginosa* ATCC 27853. Drugs were administered 4 times at q2h and burdens measured 9h post infection.



Loewe additivity interaction index

Effect on Burden	Combination 1:1	Combination 2:1	Combination 4:1
1.0 log_{10} below vehicle	0.99	0.73	0.35
Stasis	0.57	0.43	0.2
1.0 log_{10} below stasis	0.53	0.61	0.31

Figure 3. Dose response curves and Loewe Additivity Interaction Index for thigh burdens at 9h post-infection with *E. coli* IR3. Drugs were administered 4 times at q2h and burdens measured 9h post infection.



Loewe additivity interaction index

Effect on Burden	Combination 1:1	Combination 2:1	Combination 4:1
1.0 log_{10} below vehicle	1.21	1.09	1.22
Stasis	0.78	0.77	1.04
1.0 log_{10} below stasis	0.5	0.63	0.61
2.0 log_{10} below stasis	0.23	0.3	0.38

Table 2. ED_{50} values of meropenem and BAL30072 alone or in combination.

		ED_{50} (mg/kg total dose)		
		K. pneumoniae ATCC BAA1705	*P. aeruginosa* ATCC 27853	*E. coli* IR3E
Meropenem monotherapy		819	297	813
BAL30072 monotherapy		608	328	323
Meropenem	MEM:SFM **1:1**	377	111	119
	MEM:SFM **1:1**	370	137	195
	MEM:SFM **1:1**	468	144	376
BAL30072	MEM:SFM **1:1**	377	111	119
	MEM:SFM **1:1**	185	69	97
	MEM:SFM **1:1**	117	35	94
Likely driver of enhanced efficacy		BAL30072	Meropenem	BAL30072

ED_{50} values were determined by curve fitting and are influenced by the magnitude of effect.

Conclusions

- Administration of meropenem and BAL30072 in combination increased the treatment efficacy window against all strains suggesting a synergistic interaction .
- The largest improvements in efficacy were observed where the isolate was resistant to one of the two monotherapies.
- The antimicrobial activity is driven predominantly by one agent of the combination and that can be predicted using *in vitro* synergy assays.
- These data suggest the combination of meropenem and BAL30072 could be an effective treatment option for MDR Gram-negative bacteria.

References

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