C-1371

Bactericidal Activity of BAL30072 Alone and in Combination with Carbapenems Against Gram-Negative Bacteria

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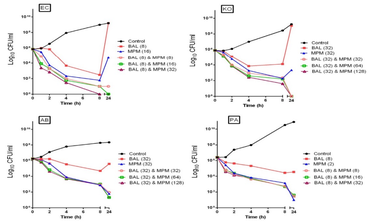
Revised Abstract

Objectives: BAL30072 (BAL) is a new monosulfactam, currently in early clinical development, that has previously been shown to have good *in vitro* activity against multi-resistant Gram-negative bacteria (GNB) and improved activity in combination with carbapenems (CBP). This study investigated the bactericidal effect of combining BAL with CBP against GNB.

Methods: Bactericidal activity was determined over 24h at 4x MIC (or fixed at 32 µg/ml if MIC ≥8 µg/ml) for BAL, meropenem (MPM), imipenem and doripenem plus 1:1, 2:1 and 4:1 CBP:BAL ratios against recent clinical isolates: 2 CBP-resistant (CBP-R) *Escherichia coli* (EC), 1 CBP-R *Klebsiella oxytoca* (KO), 2 CBP-R *K. pneumoniae*, 2 CBP-R *A. baumannii*, 2 CBP-R *P. aeruginosa* (PA) and the control PA ATCC27853.

Results: Improved bactericidal activity was observed against the EC, KO & KP when BAL was combined with the three CBPs. Re-growth occurred with BAL and/or CBPs alone at 24h but not in combination. Kill curves with PA or AB did not show this consistent 'synergy' but generally activity in combination was better than BAL alone. Example kill curves for BAL & MPM against one isolate per genus are given in the Figure (number in parenthesis = test concentration µg/ml).



Conclusions: These data, consistent with previous MIC studies [ECCMID 2014, P-0296], support the hypothesis that combining BAL with a CBP could translate into a therapeutic advantage.

Introduction

BAL30072 is a novel monosulfactam antibiotic (Figure 1) with potent antimicrobial activity against a broad range of Gram-negative bacteria, including clinically increasingly problematic multidrug-resistant pathogens such as *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Klebsiella* spp. and *Enterobacter* spp . BAL30072 is stable towards many types of beta-lactamases that can deactivate most of the currently marketed beta-lactam antibiotics such as cephalosporins and carbapenems. The compound is taken up very readily into bacteria, exploiting essential nutrient uptake systems and is able to circumvent resistance caused by changes in the outer membrane of Gram-negative bacteria. In experimental settings, bacterial resistance towards BAL30072 develops more slowly than it does to other drugs. The compound has shown to be highly compatible with agents used for treating Gram-positive infections. Preliminary data suggest BAL30072 may act synergistically with some agents used for treating Gram-negative infections, such as carbapenems (1).

The *in vitro* bactericidal activity of BAL30072 alone and in combination with meropenem, imipenem or doripenem was investigated against clinical isolates of *Enterobacteriaceae* and non-fermentors.

Materials & Methods

Isolates: 2 carbapenem-resistant *Escherichia coli*, 1 carbapenem-resistant *Klebsiella oxytoca*, 2 carbapenem-resistant *K. pneumoniae*, 2 carbapenem-resistant *A. baumannii*, 2 carbapenem-resistant *P. aeruginosa* and the control *P. aeruginosa* ATCC27853 were used. Isolates used in the current study and MIC values are listed in Table 1.

Susceptibility and bactericidal activity: MICs were performed by broth microdilution as per CLSI guidelines and interpretations (2, 3). Bactericidal activity was determined over 24h at 4x MIC (or fixed at 32 µg/ml if MIC ≥8 µg/ml) for BAL30072, meropenem (MPM), imipenem and doripenem plus 1:1, 2:1 and 4:1 carbapenem:BAL30072 ratios.

Results

Figure 1: Chemical structure of BAL30072

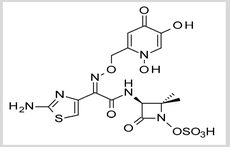


Figure 2. Bactericidal activity of BAL30072 and meropenem alone and combined against *E. coli* 848705
[BAL30072 MIC = 2 µg/ml; Meropenem MIC = 4 µg/ml]

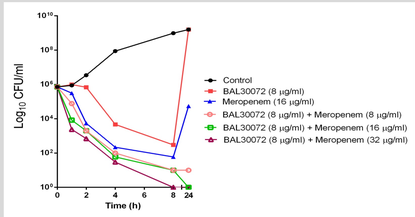


Figure 3. Bactericidal activity of BAL30072 and meropenem alone and combined against *K. pneumoniae* 857973
[BAL30072 MIC = 0.25 µg/ml; Meropenem MIC > 32 µg/ml]

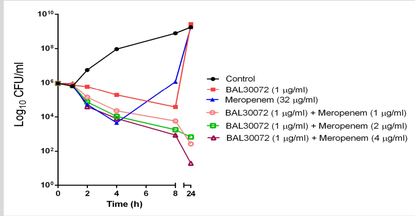


Figure 4. Bactericidal activity of BAL30072 and meropenem alone and combined against *P. aeruginosa* 867846
[BAL30072 MIC = 0.25 µg/ml; Meropenem MIC = 8 µg/ml]

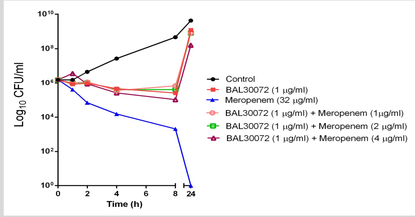


Figure 5. Bactericidal activity of BAL30072 and meropenem alone and combined against *A. baumannii* 863842
[BAL30072 MIC = 2 µg/ml; Meropenem MIC >32 µg/ml]

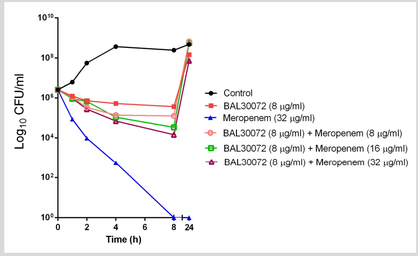


Figure 6. Bactericidal activity of BAL30072 and meropenem alone and combined against *A. baumannii* 846518
[BAL30072 MIC = 16 µg/ml; Meropenem MIC = 32 µg/ml]

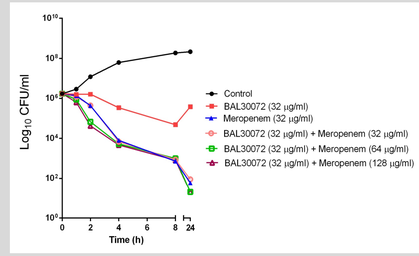


Figure 7. Bactericidal activity of BAL30072 and meropenem alone and combined against *P. aeruginosa* 850688
[BAL30072 MIC >32 µg/ml; Meropenem MIC >32 µg/ml]

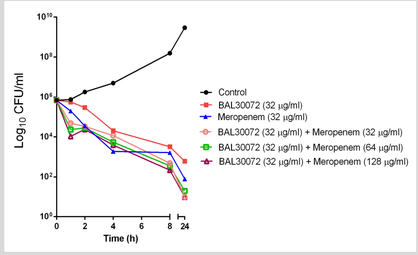


Figure 8. Bactericidal activity of BAL30072 and meropenem alone and combined against *P. aeruginosa* ATCC 27853
[BAL30072 MIC = 2 µg/ml; Meropenem MIC = 0.5 µg/ml]

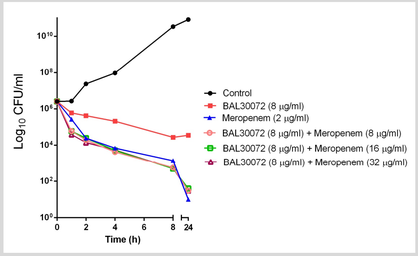
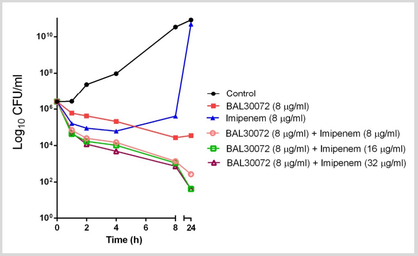


Figure 9. Bactericidal activity of BAL30072 and imipenem alone and combined against *P. aeruginosa* ATCC 27853
[BAL30072 MIC = 2 µg/ml; Imipenem MIC = 2 µg/ml]



Results

- Time kill curves for BAL30072 alone or in combination with meropenem against *E. coli* 848705 and *K. pneumoniae* 857973 are shown in Figs 2 and 3.
- In both cases, despite initial bactericidal activity, re-growth occurred with either meropenem alone or BAL30072 alone. However, the combination of meropenem and BAL30072 not only prevented this re-growth but also enhanced overall bactericidal activity. This also occurred with the other *E. coli* and *Klebsiella* spp. investigated (data not shown). Similar results were also seen with doripenem and imipenem against the *Enterobacteriaceae* (data not shown).
- When *A. baumannii* and *P. aeruginosa* were tested the results were strain and carbapenem-specific.
- For *A. baumannii* 863842 and *P. aeruginosa* 867846 re-growth occurred with BAL30072 and BAL30072:meropenem combinations but not with meropenem alone (Figs 4 & 5). With doripenem and imipenem, re-growth occurred with BAL30072:carbapenem combinations and with carbapenem alone (data not shown).
- For *A. baumannii* 846518, BAL30072 did not affect the activity of meropenem (Fig. 6) or the other carbapenems tested (data not shown). This was also the case with *P. aeruginosa* 850688 (Fig. 7).
- For *P. aeruginosa* ATCC27853, combination of BAL30072 with meropenem had no effect (Fig. 8) (as also seen with doripenem - data not shown), but combination with imipenem prevented re-growth (Fig. 9).

Table 1: Susceptibility of Isolates Used in the Study

Organism Name	IHMA #	MIC (µg/ml):			
		BAL30072	Doripenem	Imipenem	Meropenem
E. coli	848705[a]	2	2	8	4
E. coli IRE 3	1042934	0.5	32	32	>32
K. oxytoca	871516[a]	4	1	32	32
K. pneumoniae	857973[a]	0.25	>32	>32	>32
K. pneumoniae BAA1705	1042935	2	16	>32	>32
P. aeruginosa ATCC 27853	NA	2	0.5	2	0.5
P. aeruginosa	867846	0.25	8	16	8
P. aeruginosa	850688	>32	>32	>32	>32
A. baumannii	846518[a]	16	32	>32	32
A. baumannii	863842[a]	2	>32	32	>32

[a]Beta-lactamase enzymes have been characterized for these strains: 848705 (TEM-1(2b), CTX-M1 & KPC-2); 871516 (SHV-12(2be) & KPC-2); 857973 (SHV-11(2b), TEM-1(2b), CTX-M15 & NDM-1); 846518 (OXA-23(c)) and 863842 (OXA-23(c)).

Conclusions

- **Clear synergy was observed by combining BAL30072 with carbapenems against the five *Enterobacteriaceae* investigated. These data suggest there is a therapeutic advantage to be obtained by combining BAL30072 with carbapenems against carbapenem-resistant *Enterobacteriaceae*. Data shown is for combinations with meropenem but combination with imipenem and doripenem had similar profiles (data not shown)**
- **For *P. aeruginosa* or *A. baumannii* the combination of BAL30072 with carbapenems did not consistently show the 'synergistic' effect as seen with the *Enterobacteriaceae*. Previous MIC studies showed improved carbapenem susceptibility (from 14% to 75%) due to 1:1 combination with BAL30072 with 100 *P. aeruginosa* and 95 *A. baumannii* (1) indicating that the degree of synergy is strain, panel and test condition dependent.**
- **These data, consistent with previous MIC studies (1), support the hypothesis that combining BAL30072 with a carbapenem could translate into a therapeutic advantage.**

References

1. Morrissey I.; Siegmund C.; Genet E.; Neri M.; Hawser S.; Jones M.; Page M.; Santerre Henriksen A. (2014). Activity of BAL30072 alone and in combination with carbapenems against Gram-negative bacteria. ECCMID 2014, abst. P0296.
2. CLSI, 2012. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Eighth Edition M07-A9. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA 19087-1898 USA.
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Acknowledgements

This project has been funded in whole or in part with Federal funds from the Department of Health and Human Services; Office of the Assistant Secretary for Preparedness and Response; Biomedical Advanced Research and Development Authority (BARDA) under Contract No. HHSO100201300010C.