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A Monobactam Plus Double β-lactamase Inhibitor Combination Designed to Overcome Multiple Resistance in Gram-Negative Bacilli: Proof of Concept in an *in vitro* Pharmacokinetic Model

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Abstract

Background: Antibiotic resistance in *P. aeruginosa* and Enterobacteriacae represent a major healthcare challenge, especially in hospitals. New approaches to overcoming resistance in Gram-negative rods (GNB) is drug development priority. A triple β-lactam combination (BLC) of BAL 0019764 (a monobactam) + BAL 0029880 (a bridged monobactam, class C β-lactamase inhibitor) + BAL 0017708 (a class A β-lactamase inhibitor) was tested at likely human free drug serum concentrations in an *in vitro* pharmacokinetic (PK) against nine GNB strains.

Methods: A hollow fibre dialysis in vitro PK model was used with an initial inoculum of 106 CFU/ml in 25% Muller-Hinton Broth. Three strains of P.aeruginosa, 3 ESBL producing Enterobacteriacae, 1 AmpC producing *Enterobacter* spp were used. GNB were multiresistant to β-lactams, amikacin and ciprofloxacin. Fully sensitive control *P. aeruginosa* or *E. coli* were used. The BLC was in a 1:1:1 ratio with a simulated Cmax of 50mg/L, half-life of 1.5h and administered 8hly for 24h. Antibacterial effect (ABE) was measured by log change in viable count before each dose and the area-under-the-bacterial kill cure 0-24h (AUBKC 24).

Results: The three *P. aeruginosa* strains have BLC MIC of < 3mg/L producing T>MIC of > 77%. The log reduction at 8h (d8), 16h (d16) and 24h (d24) were: 3.4 + 0.9; 3.9 + 0.9; and 3.6 + 0.9 respectively. The AUBKC ratio BLC/control was 0.16 + 0.04. For the ESBL/AmpC producers the d8, d16 and d24 were: 3.9 + 0.9; 4.2 + 0.6; and 4.0 + 0.6 respectively the BLC/control ratio was 0.11 + 0.06. BLC had a similar ABE against the susceptible control strains tested.

Conclusions: BLC has a significant ABE against β-lactam resistant *P. aeruginosa* and ESBL/AmpC producing Enterobacteriacae at likely human serum concentrations. Triple β-lactam combinations may represent a way of overcoming clinically important resistance in GNBs.

Introduction

- A variety of β-lactam antibiotic/β-lactamase inhibitor combinations have been studied *in vitro* to ascertain the optimum combination providing potency against Enterobacteriaceae, *Acinetobacter* spp, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Burkholderia cepecia*.
- Previous data has shown that the β-lactam antibiotic/β-lactamase inhibitor combination BAL0019764-000-005, BAL0029880-000-003 and BAL0017708-001-001 (BAL30376) in a 1:1:1 ratio produced MICs in the range 0.12->64 mg/L against strains with defined resistance mechanisms.
- In this study we tested BAL30376 against *P. aeruginosa* (3), *Enterobacter cloacae* (2) and a *Klebsiella pneumoniae* containing ESBLs, an *Enterobacter cloacae* containing an AmpC β-lactamase, and two ATCC control strains of *E. coli* and *P. aeruginosa* in an *in vitro* pharmacokinetic model.

Materials and Methods

- The following strains were used; *P. aeruginosa* 35019, *P. aeruginosa* 35020, *P. aeruginosa* 35021; *Ent.cloacae* 33109 (ESBL +ve), *Ent.cloacae* 32296 (ESBL +ve), Kl.pneumoniae 6673 (ESBL +ve), *Ent.cloacae* 29553 (AmpC), *E.coli* ATCC 25922 and *P. aeruginosa* ATCC 25783. An inoculum of 10^6cfu/mL was used
- The free drug pharmacokinetics simulated for BAL30376 were; C_{max} 50mg/l, serum half-life 1.5h administered 8hrly. BAL was added to the chamber at T0, T8 and T16. The targeted pharmacokinetic profile was confirmed using vancomycin and FPIA assay.
- An in vitro hollow fibre pharmacokinetic model system consisting of 3 bioreactors using 25% Mueller Hinton broth was utilised. An inoculum of 10^6cfu/mL was used throughout.
- Samples were collected from the model throughout the 24h time period (T0, 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24h) for assessment of viable count and determination of drug concentrations. Bacterial counts were determined by plating neat and 10^{-3} dilutions in sterile saline onto nutrient agar plates using a spiral plater. The limit of detection was 2.0x 10^2cfu/mL.
- The antibacterial effect measures calculated for each inoculum were; log change in viable count at 8,16 and 24 (Δ24) hours; the area-under-the bacterial kill curve 0-24h (AUBKC24). All experiments were performed in singlicate.

Results

- The antibacterial effect of BAL on the test strains is shown on the Table and Figures 1-3.
- Two of the *P. aeruginosa* strains (35021 and 35019) were rapidly killed with a 3 log drop noted at 6h and viable counts were below the limit of detection at 5 and 12h respectively. *P. aeruginosa* 35020 showed a 6log reduction in viable count at 4h but regrowth occurred at 5h and was maintained throughout the remainder of the simulation. These were results were reflected in the AUBKC values (Table and Figure 1). The *P. aeruginosa* ATCC 25783 strain was eradicated from the model at 12h.
- Ent.cloacae strains showed a similar pattern of killing with viable counts below the limit of detection at 8h. The AmpC containing *Ent.cloacae* was also below the limit of detection at 8h but some regrowth was observed at 24h (Figure 2).
- *Kl.pneumoniae* (ESBL +ve) showed a similar pattern of killing to the *Ent.cloacae* strains. The E.coli ATCC 25922 was rapidly killed and eradicated from the model at 3h (Figure 3)

Conclusions

- BAL30376 has a significant antibacterial effect at likely human serum concentrations against clinically important strains such as β-lactam resistant *P. aeruginosa* and ESBL/AmpC producing Enterobacteriacae.
- Triple β-lactam combinations may represent a way of overcoming clinically important resistance in Gram-negative bacilli.

Table 1: The antibacterial effect measures of BAL30376 against the test strains

strain	BAL MIC	T>MIC	log red. in viable count			AUBKC24 (log.cfu/ml.h)
			8h	16h	24h	
P. aeruginosa 35019	3.0	77	3.6	4.0	4.0	15.4
P. aeruginosa 35020	1.0	100	2.0	3.8	2.4	30.8
P. aeruginosa 35021	0.9	100	4.6	4.6	4.6	25.9
Kl.pneumoniae 6673	1.0	100	4.4	4.46	4.4	14.4
Ent. cloacae 33109	1.2	100	4.4	4.4	4.4	13.9
Ent. cloacae 30647	4.0	70	4.3	4.6	3.1	14.9
Ent. cloacae 32296	0.7	100	2.5	3.3	3.9	30.7
E. coli ATCC 25922	0.12	100	3.6	4.2	4.2	7.6
P. aeruginosa ATCC 25783	2.6	81	2.5	3.3	3.9	11.5

Figure 1: Effect of BAL against *P. aeruginosa* spp 35019, 35020 and 35021 and *P. aeruginosa* ATCC25783

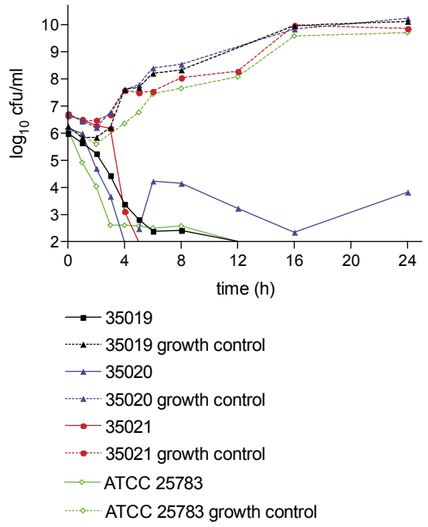

Figure 2: Effect of BAL 30376 against *Ent. cloacae* strains 33109, 30647 and 32296

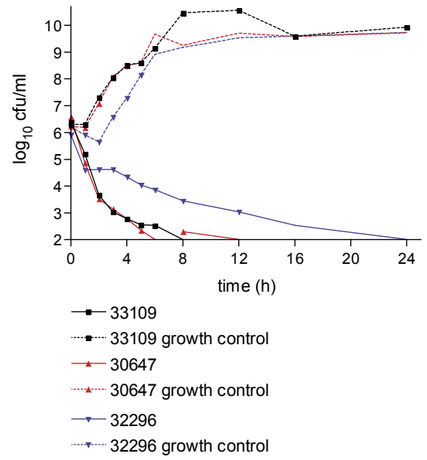

Figure 3: Effect of BAL 30376 against *Kl. pneumoniae* and E. coli ATCC25922