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In vitro activity of BAL30072 against *Burkholderia pseudomallei*

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Abstract

Burkholderia pseudomallei is an intrinsically antibiotic-resistant Category B priority pathogen and the aetiological agent of melioidosis. Treatment of *B. pseudomallei* infection is biphasic and lengthy in order to combat the acute and chronic phases of the disease. Acute-phase treatment preferably involves an intravenous cephalosporin (ceftazidime) or a carbapenem (imipenem or meropenem). In this study, the anti-*B. pseudomallei* efficacy of a new monosulfactam, BAL30072, was tested against laboratory strains 1026b and 1710b and several isogenic mutant derivatives as well as a collection of clinical and environmental *B. pseudomallei* strains from Thailand. More than 93% of the isolates had minimal inhibitory concentrations (MICs) in the range 0.004–0.016 µg/mL. For the laboratory strain 1026b, the MIC of BAL30072 was 0.008 µg/mL, comparable with the MICs of 1.5 µg/mL for ceftazidime, 0.5 µg/mL for imipenem and 1 µg/mL for meropenem. Time–kill curves revealed that BAL30072 was rapidly bactericidal, killing >99% of bacteria in 2 h. BAL30072 activity was not significantly affected by efflux, it was only a marginal substrate of PenA β-lactamase, and activity was independent of malleobactin production and transport and the ability to transport pyochelin. In summary, BAL30072 has superior in vitro activity against *B. pseudomallei* compared with ceftazidime, meropenem or imipenem and it is rapidly bactericidal.

Keywords

Burkholderia pseudomallei; Melioidosis; Therapy; Monosulfactam; Efflux; Siderophore

1. Introduction

Burkholderia pseudomallei is the aetiological agent of melioidosis, a rare but serious and emerging tropical disease [1]. In the USA, the bacterium is also a Category B priority and Select Agent pathogen because of its potential use as biological weapon [2]. The current treatment regimen for human melioidosis is intravenous administration of ceftazidime or a

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carbapenem (imipenem or meropenem) for at least 10–14 days, followed by oral eradication therapy using trimethoprim/sulfamethoxazole with or without doxycycline for 12–20 weeks [3,4]. For patients who cannot tolerate this therapy or where it is contraindicated (e.g. children and pregnant women), the choice of oral therapy is amoxicillin/clavulanic acid (AMC). In a quest to increase the arsenal of therapeutics for melioidosis, the anti-*B. pseudomallei* efficacy of a new monosulfactam BAL30072 (Fig. 1) was tested, which has shown especially potent in vitro activity against Gram-negative bacteria that have acquired the reputation of 'superbugs' because of their high levels of resistance to many of the currently marketed antibiotics [5,6].

2. Materials and methods

2.1. Bacterial strains

The *B. pseudomallei* laboratory strains as well as their isogenic derivatives used in this study are listed in Table 1. The study also included an additional 30 clinical and 30 environmental isolates from Thailand. Strain 1026b was originally isolated from a patient in Ubon Ratchathani in Northeast Thailand and is one of the widely used laboratory prototype strains [7].

2.2. Susceptibility testing

Susceptibility was tested by determining minimal inhibitory concentrations (MICs). MICs for BAL30072 and carbenicillin were determined using the two-fold broth microdilution technique [8] with Difco™ Mueller–Hinton broth (Becton Dickinson, Franklin Lakes, NJ) as the growth medium. Etest strips (AB bioMérieux, Marcy l'Étoile, France) were used to determine MICs for ceftazidime, amoxicillin, AMC, imipenem and meropenem. For time–kill curves, ca. 10^6 log-phase cells (optical density at 600 nm 0.5–0.7) of strain 1026b was inoculated in Luria–Bertani Lennox (LB) broth (MO BIO Laboratories, Carlsbad, CA, USA) with and without BAL30072. The cultures were shaken at 37 °C, aliquots were removed at regular time intervals and viable bacteria were determined by plating 10-fold serial dilutions on LB agar plates. Time–kill curves were performed at $1\times$ (0.008 µg/mL) and $4\times$ (0.032 µg/mL) MIC.

3. Results and discussion

3.1. Susceptibility of strain 1026b

Susceptibility of the clinical isolate, and now commonly used laboratory strain, 1026b [7] to BAL30072 was initially tested and was compared with that of other β -lactams (Table 2). The MIC for BAL30072 was 0.008 µg/mL, comparable with the MICs of 1.5 µg/mL for ceftazidime, 2 µg/mL for AMC, 0.5 µg/mL for imipenem and 1 µg/mL for meropenem, all of which are used or have been tested for treatment of human melioidosis. The in vitro activity of BAL30072 is thus superior to these clinically used antibiotics. Time–kill curves revealed that BAL30072 was rapidly bactericidal, killing >99% of bacteria in 2 h (data not shown).

3.2. Susceptibility of clinical and environmental strains

Next, the susceptibility of a panel of randomly chosen 30 clinical (plus strain 1026b) and 30 environmental isolates was tested. The distribution of BAL30072 MICs for the 61 strains tested was 7 isolates (11.5%) at 0.004 µg/mL, 24 isolates (39.3%) at 0.008 µg/mL, 26 isolates (42.6%) at 0.016 µg/mL, 3 isolates (4.9%) at 0.032 µg/mL and 1 isolate (1.6%) at 1 µg/mL (Fig. 1). More than 93% of the isolates had MICs in the range 0.004–0.016 µg/mL. Clinical and environmental strains exhibited similar susceptibility profiles.

3.3. Effects of chromosomally-encoded resistance determinants on BAL30072 susceptibility

Very little is known about antibiotic resistance in *B. pseudomallei* and the only two mechanisms that have been characterised in some detail are the PenA β -lactamase [10,11] and efflux [12,13]. Thus, the effects of β -lactamase and efflux on susceptibility to BAL30072 were tested. Like imipenem and meropenem, BAL30072 is only weakly hydrolysed by the chromosomally-encoded PenA class A β -lactamase (MICs for 1026b and the *penA* mutant Bp319 were 0.008 μ g/mL and 0.002 μ g/mL, respectively) (Table 2). These observations were not due to lack of PenA expression in 1026b because this strain expresses PenA and was highly resistant to amoxicillin and carbenicillin, two known PenA substrates. Deletion of *penA* in Bp319 resulted in significantly increased susceptibility to amoxicillin and carbenicillin, but not to BAL30072, ceftazidime, imipenem and meropenem. BAL30072 activity was not affected by efflux since the MICs observed with 1026b and its $\Delta(amrRAB-oprA)$ derivative Bp50 were the same (0.008 μ g/mL).

3.4. High-affinity iron transporters are not required for efficient BAL30072 activity

The siderophore monosulfactam BAL30072 was designed to act like a 'Trojan horse' by exploiting iron uptake systems to gain access to its target. Presence of the catechol bioisosteric moiety is thought to facilitate ferric iron binding. The BAL30072- Fe^{3+} complex could then potentially be actively transported across the outer membrane by a cognate outer membrane protein to gain access to the periplasm. The activity of BAL30072 against mutants Bp327, Bp338, Bp414 and Bp415 that were defective in malleobactin and/or pyochelin biosynthesis and transport was therefore tested. As all four mutants exhibited susceptibilities that were similar to the prototype strains 1026b and 1710b, it was suggested that the high-affinity malleobactin and pyochelin receptors/transporters FmtA and FptA, respectively, are not required for efficient BAL30072 uptake. The lack of effect of impaired production or transport of malleobactin and pyochelin on BAL30072 is not unexpected owing to structural differences between siderophore moieties. Malleobactin is a hydroxamate siderophore [14] and pyochelin is a phenolate siderophore [15]. One would not expect the defect in a hydroxamate system to affect a catechol, and pyochelin is not good analogue of the catechol either.

4. Conclusion

It was previously noted that the unique pattern of penicillin-binding protein inhibition and bactericidal mode of action of BAL30072 confer potent in vitro activity against Gram-negative bacteria [5]. In this report, it was shown that BAL30072 has superior in vitro activity against *B. pseudomallei* compared with ceftazidime, meropenem and imipenem, as well as being rapidly bactericidal. It was not possible to attribute the superior activity of BAL30072 to facilitated cellular uptake via any of the currently characterised iron uptake systems of *B. pseudomallei*.

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Fig. 1. Minimal inhibitory concentration (MIC) distribution of BAL30072 in a panel of 61 *Burkholderia pseudomallei* strains. The MIC₅₀ (MIC for 50% of the strains) and MIC₉₀ (MIC for 90% of the strains) are indicated by arrows.

Table 1**Bacterial strains used in this study**

Strain	Relevant genotype	Reference/source
1026b	Prototype strain; clinical isolate	[7]
1710b	Clinical isolate	S. Peacock
708a	Clinical isolate; contains a >140 kb deletion that is fusing the equivalents of 1710b <i>BURPPS1710b_2155</i> and <i>BURPPS1710b_2054</i> genes; natural malleobactin biosynthesis gene cluster mutant	[9]
Bp50	1026b with $\Delta(amrRAB-oprA)$; AmrAB-OprA efflux pump mutant	This study
Bp319	1026b with $\Delta penA$; PenA β -lactamase deletion mutant	This study
Bp327	1710b with $\Delta(mbaS-mbaF)$; malleobactin biosynthesis gene cluster mutant	This study
Bp338	1710b with strain 708a $\Delta(BURPPS1710b_2155-BURPPS1710b_2054)$; malleobactin biosynthesis gene cluster mutant	This study
Bp414	1710b with $\Delta fptA$; pyochelin receptor mutant	This study
Bp415	1710b with $\Delta(mbaS-mbaF) \Delta fptA$; malleobactin biosynthesis gene cluster and pyochelin receptor mutant	This study

Table 2

Susceptibilities of selected *Burkholderia pseudomallei* strains

Strain ^a	MIC (μg/mL) ^b					
	BAL30072	Ceftazidime	Amoxicillin	AMC	Imipenem	Meropenem
1026b	0.008	1.5	>256	2	0.5	1
1710b	0.016	1.5	256	1.5	0.38	0.75
Bp50 Δ(<i>amrRAB-oprA</i>)	0.008	3	>256	2	0.75	1.0
Bp319 Δ <i>penA</i>	0.002	1.0	2	1.5	0.25	1
Bp327 Δ(<i>mbaS-mbaF</i>)	0.008	1.5	>256	1.5	0.38	1
Bp338 Δ(<i>BURPPS1710b_2155-BURPPS1710b_2054</i>) ^c	0.016	1.5	>256	1.5	0.38	0.75
Bp414 Δ <i>pntA</i>	0.016	N/D	N/D	N/D	N/D	N/D
Bp415 Δ(<i>mbaS-mbaF</i>) Δ <i>pntA</i>	0.008	N/D	N/D	N/D	N/D	N/D

MIC, minimal inhibitory concentration; AMC, Amoxicillin/clavulanic acid; N/D, not determined.

^aBp50 and Bp319 are derivatives of 1026b; and Bp327, Bp338, Bp414 and Bp415 are derived from 1710b (see Table 1).

^bMIC determinations were performed using Etest strips, except for BAL30072 and carbenicillin that were determined using the microbroth dilution method. Although carbenicillin is no longer used in the clinic, it was included because it is a good substrate for PenA β-lactamase and an indicator for its expression.

^cBp338 contains the same large (>140 kb) Δ(*BURPPS1710b_2155-BURPPS1710b_2054*) as the natural malleobactin siderophore deletion mutant 708a [9] that exhibits the same BAL30072 susceptibility.