In Vitro Activity of Cefepime-AAI101 towards *Burkholderia mallei*

Poster 99

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Background

AAI101 (Figure 1) is a novel extended-spectrum β-lactamase inhibitor with potent activity against ESBLs. AAI101 in combination with cefepime, a fourth-generation cephalosporin, has completed Phase II clinical trials in patients with complicated urinary tract infections. Cefepime-AAI101 is developed for the treatment of serious Gram-negative infections caused by ESBL-producing Enterobacteriaceae and other Gram-negative pathogens.

This study sought to assess the *in vitro* activity of cefepime-AAI101 and comparators towards clinical isolates of *Burkholderia mallei* (Figure 2), the etiological agent of glanders.

B. mallei is endemic to Asia, Africa, the Middle East, and Central and South America and is highly infectious in aerosolized form. It was used to infect animals and humans during World War I (1), and was a subject of biowarfare research during World War II and the Soviet invasion of Afghanistan (2). There is no vaccine against glanders, no validated serologic test for human glanders, and antibiotic options for *B. mallei* infection are poorly explored; no antimicrobials for postexposure prophylaxis or treatment for this pathogen are FDA-approved. The United States Centers for Disease Control and Prevention classifies *B. mallei* as a Tier 1 Select Agent (3).

Resistance to β-lactam antibiotics by *B. mallei* is attributed to the presence of an ESBL, "Pen I" (sometimes referred to as "PenA") (4,5). The MIC_{90} of *B. mallei* towards amoxicillin is lowered from 128 µg/mL to 8 µg/mL by addition of clavulanic acid (6; also see ref. 7).

Methods

MICs were determined for 30 geographically diverse strains of *B. mallei*. This collection provides a good representation of isolates likely to be encountered anywhere the world; performance of a drug against an unknown isolate of *B. mallei* is expected to fall within the MIC ranges obtained for this pathogen panel.

B. mallei was cultured on Trypticase Soy agar (35°C, 16-24 h, ambient air), and colonies suspended in cation-adjusted Mueller-Hinton broth (CAMHB) to a density of 10^5 CFU/ml (0.5 McFarland standard). Twofold dilutions of test compounds (AAI101, cefepime, cefepime-AAI101 [fixed at 1 µg/mL and 2 µg/mL], and azithromycin) were prepared in CAMHB. Fifty µL each of cell suspension and diluted test compound were added to wells in 96-well microtiter plates, and broth microdilution MICs determined following CLSI protocols (9) over a concentration range 0.03-64 µg/mL for the test compounds. Positive controls (no test compound) and negative controls (no inoculum) were included in each plate during each assay run. Plates were incubated in ambient air at 35°C, and MICs determined by visual inspection after 18-24 h.

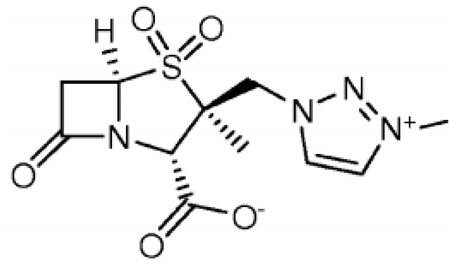

Figure 1. Chemical structure of AAI101

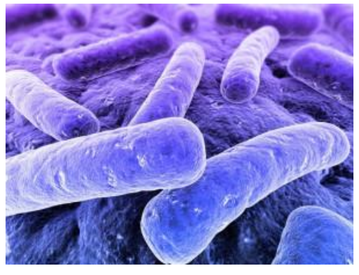

Figure 2. False-color electron micrograph of *B. mallei*

Results

AAI101 alone was modestly inhibitory towards *B. mallei*, with an MIC_{90} (n = 30) of 16 µg/mL (Table). AAI101 has been noted previously to have some inhibitory activity towards meropenem-susceptible isolates of *Acinetobacter baumannii* (10), though alone it is inactive against Enterobacteriaceae (11).

Azithromycin was selected as the comparator agent; though no antimicrobial for this pathogen is FDA-approved, Hershfield *et al.* (8) demonstrated that this macrolide promotes survival and splenic clearance in a murine *B. mallei* whole body aerosol model using both post-exposure prophylaxis and treatment of active infection.

The co-presence of AAI101 at a low concentration had a profound impact on the *in vitro* susceptibility of *B. mallei* to cefepime (Table). Whereas the MIC_{90} for this pathogen towards cefepime alone exceeded 16 µg/mL, addition of as little as 1 µg/mL of AAI101 diminished the MIC_{90} of cefepime by ≥8 $\log_2$ dilution steps. MIC_{50} and MIC_{90} values for cefepime-AAI101 were both eight-fold lower than those obtained for azithromycin.

Table. *In vitro* efficacies of cefepime-AAI101 and comparators towards a geographically diverse panel of *B. mallei* (n = 30).

Drug	MIC range (µg(mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
AAI101	2-64	8	16
Cefepime	4-≥16	16	>16
Cefepime-AAI101 (2)*	≤0.03-0.25	0.06	0.125
Cefepime-AAI101 (1)**	≤0.03-0.25	0.06	0.125
Azithromycin	0.125-2	0.5	1

*Cefepime + fixed AAI101 concentration of 2 µg/mL; ** Cefepime + fixed AAI101 concentration of 1 µg/mL

Conclusions

- Addition of ≤1 µg/mL of AAI101, a novel extended-spectrum β-lactamase inhibitor, reduced the MIC of cefepime towards *Burkholderia mallei*, a CDC Category B pathogen and causative agent of glanders, by ≥8 $\log_2$ dilution steps.
- Cefepime-AAI101 proved more efficacious *in vitro* than azithromycin, the U. S. Army's proposed candidate for post-exposure prophylaxis and treatment of active infection by *Burkholderia mallei*.
- Cefepime-AAI101 is developed for the treatment of infections caused by ESBL-producing Enterobacteriaceae and other Gram-negative pathogens. These results indicate that cefepime-AAI101 is also a promising candidate for development as a treatment for *Burkholderia mallei* exposure/infection under "animal rule" guidelines (12).

References

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