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Activity of a novel extended-spectrum β-lactamase inhibitor, AAI101, combined with cefepime against β-lactamase-producing Enterobacteriaceae in a murine septicemia model

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ABSTRACT

Background: AAI101 is a novel β-lactamase inhibitor with potent activity against ESBLs and other β-lactamases. AAI101 in combination with cefepime is in development for therapy of serious Gram-negative infections, and has completed Phase 2 clinical trials. This study examined the ability of AAI101 to restore cefepime activity against cefepime-resistant Enterobacteriaceae in a mouse model of septicemia.

Materials/methods: Immunocompetent female RjOrl:SWISS mice (6 animals per cohort) were injected intraperitoneally with lethal doses of Enterobacteriaceae clinical isolates producing one or more ESBLs together with an AmpC β-lactamase or OXA-48 (cefepime-resistant), or with *Escherichia coli* ATCC 25922 (cefepime-susceptible). At 1 h and 4 h post-infection, mice were dosed subcutaneously with vehicle, cefepime, cefepime/AAI101 (2/1 $^{w}/_{w}$), or meropenem. Drugs were administered at increasing half-log concentrations over the range 0.1-300 mg/kg (cefepime-resistant strains) or 0.01-30 mg/kg (*E. coli* ATCC 25922). Animal survival was recorded three times daily for 7 days, and the protective dose required for 50% survival (PD_{50}) calculated using Reed and Muench's cumulative distribution function.

Results: AAI101 restored the *in vitro* activity of cefepime in strains coproducing one or more ESBLs and an AmpC β-lactamase or OXA-48 carbapenemase. Infected animals treated with vehicle succumbed within 24 h of infection. Cefepime monotherapy was effective only in mice infected with the non-ESBL-producing strain *E. coli* ATCC 25922. In animals infected with cefepime-resistant isolates, AAI101 lowered PD_{50}s >10-fold when administered in combination with cefepime compared to cefepime alone, and PD_{50}s were similar for all three cefepime-resistant strains. Cefepime/AAI101 was comparable or superior to meropenem for the derepressed chromosomal AmpC and OXA-48 producers, whereas meropenem had a lower PD_{50} for the plasmid-encoded AmpC producer.

Conclusions: AAI101 combined with cefepime is a potent inhibitor of Enterobacteriaceae co-producing extended-spectrum β-lactamases with class C or class D β-lactamases *in vitro* and in mice with a lethal septicemia. Cefepime/AAI101 efficacy compares well with meropenem, and additionally covers OXA-48 producers. AAI101 in combination with cefepime is a promising new therapeutic modality for treatment of infections caused by drug-resistant Enterobacteriaceae.

BACKGROUND

ESBL-producing Enterobacteriaceae was recently classified by the World Health Organization as "Priority Pathogen: CRITICAL".

AAI101 is a novel extended-spectrum β-lactamase inhibitor under development in combination with cefepime for treatment of multidrug-resistant Gram-negative bacterial infections. The combination cefepime/AAI101 has completed Phase 2 clinical trials, and has been granted Qualified Infectious Disease Product and Fast Track designations by the FDA.

The objective of this study was to evaluate the activity of AAI101 combined with cefepime in a mouse model of septicemia, towards a panel of clinical Enterobacteriaceae isolates expressing ESBLs combined with other β-lactamases.

PD_{50}s were determined for cefepime alone and for cefepime/AAI101. Meropenem served as comparator.

The relative values of PD_{50} for cefepime/AAI101 and cefepime alone reflects the ability of AAI101 to restore the activity of cefepime *in vivo* from infections by β-lactamase producing bacterial pathogens.

MATERIALS AND METHODS

Bacterial strains. Clinical isolates, genotyped and well-characterized for β-lactam resistance mechanisms, were selected for evaluation in the septicemia model (**Table 1**).

Susceptibility tests. MICs for cefepime, cefepime + a fixed concentration of 8 µg/mL of AAI101, and meropenem were obtained in triplicate for the aforementioned clinical isolates by broth microdilution, as per CLSI guidelines (**Table 1**).

Septicemia infection model. Immunocompetent mice (20-24 g) were infected intraperitoneally with cell suspensions corresponding to the LD_{100} for a given strain as previously determined. Six animals were used per treatment group.

Pharmacokinetics. Cefepime and AAI101 plasma titers were determined by single-dose pharmacokinetics of cefepime/AAI101 in infected mice. Samples were collected by cardiac puncture at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h following subcutaneous administration of cefepime/AAI101 60/30 mg/kg and drug titers quantified at the Aptuit Center for Drug Discovery & Development (Verona, Italy; **Figures 1 and 2**).

Treatment. Test compounds were prepared in sterile saline (0.9%) as half-log dilutions, and administered by subcutaneous (s.c.) injection into the neck at 1 h and 4 h post-infection. For each bacterial isolate tested 25 groups were included:

- 8 treatment groups received cefepime
- 8 treatment groups received cefepime/AAI101
- 8 treatment group received meropenem
- 1 treatment group received physiological saline

Cefepime and meropenem were dosed over a range of 0.1-300 mg/kg for cefepime-resistant isolates, whereas cefepime-susceptible *E. coli* ATCC 25922 was dosed over the range 0.01-30 mg/kg.

Cefepime/AAI101 was dosed at a fixed ratio of 2/1 ($^{w}/_{w}$) over a range of 0.1-300 mg/kg for the cefepime part (cefepime-resistant isolates) or 0.01-30 mg/kg for the cefepime part (*E. coli* ATCC 25922).

Analysis. The method of Reed and Muench (cumulative distribution function) was used to calculate the PD_{50} for cefepime, cefepime/AAI101, and meropenem for each bacterial strain.

RESULTS

Table 1. Modal MICs (µg/mL) for the test strains.

Clinical isolate (β-lactamases)	Cefepime	Cefepime + 8 µg/mL AAI101	Meropenem
E. coli ATCC 25922 (low-level constitutive EC-5)	0.03	0.03	0.03
K. pneumoniae R-43 (CTX-M-15, SHV-12, DHA-1)	128	0.25	1
K. pneumoniae B-124 (CTX-M-15, OXA-48)	32	0.5	2
E. cloacae B-143 (CTX-M-15, derepressed cAmpC)	32	1	0.125

Table 2. PD_{50}s for cefepime, cefepime/AAI101, and meropenem against tested isolates.

Clinical isolate (β-lactamases)	Cefepime	Cefepime/AAI101 (2/1, $^{w}/_{w}$)*	Meropenem
E. coli ATCC 25922 (low-level constitutive EC-5)	0.05	0.06	0.23
K. pneumoniae R-43 (CTX-M-15, SHV-12, DHA-1)	>300	15.1	3.8
K. pneumoniae B-124 (CTX-M-15, OXA-48)	141	12.8	43.1
E. cloacae B-143 (CTX-M-15, derepressed cAmpC)	>300	16.0	20.3

*The PD_{50} value represents mg/kg of the cefepime component.

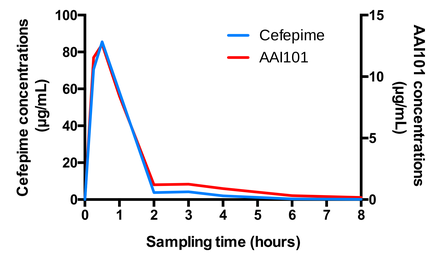

Figure 1. Cefepime and AAI101 plasma levels (µg/mL) following s.c. administration of cefepime/AAI101 60/30 mg/kg

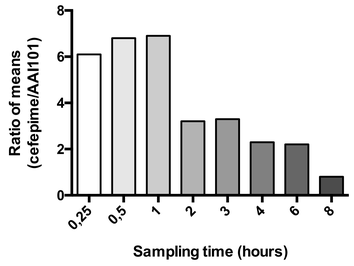

Figure 2. Cefepime/AAI101 ratio of means in mouse plasma following s.c. administration of cefepime/AAI101 60/30 mg/kg

DISCUSSION

- For all cefepime-resistant β-lactamase-producing isolates investigated in this mouse model of septicemia, AAI101 restored the efficacy of cefepime.
- Against the cefepime-resistant *Klebsiella pneumoniae* isolates expressing CTX-M-15 (ESBL) in combination with SHV-12 (ESBL) and DHA-1 (plasmidic AmpC), or CTX-M-15 in combination with OXA-48 (carbapenemase), cefepime/AAI101 proved highly efficacious.
- Against the cefepime-resistant *Enterobacter cloacae* isolate expressing CTX-M-15 in combination with a chromosomal derepressed AmpC, cefepime/AAI101 also demonstrated very good efficacy.
- No difference in cefepime and cefepime/AAI101 groups was observed for the cefepime-susceptible isolate *Escherchia coli* ATCC 25922, with negligible expression of a chromosomal AmpC (EC-5).
- Cefepime/AAI101 efficacy was comparable to that of meropenem.

CONCLUSION

Cefepime/AAI101 may offer a therapeutic alternative to carbapenems as first-line agents for treatment of infections caused by Enterobacteriaceae producing extended-spectrum β-lactamases, alone or together with other β-lactamase types.