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

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ABSTRACT

Background: AAI101 is a novel β-lactamase inhibitor with potent activity against ESBLs and other β-lactamases. AAI101 combined with cefepime has completed Phase 2 clinical trials and was granted Qualified Infectious Disease Product and Fast Track designations by the FDA. The ability of AAI101 to restore cefepime activity towards cefepime-resistant Enterobacteriaceae with defined constellations of β-lactamases was examined in a neutropenic mouse pneumonia model.

Materials/methods: MICs of test isolates were determined according to CLSI guidelines. Pharmacokinetic profiles in male ICR mice were obtained following intravenous (IV) administration of single doses of cefepime/AAI101 (60/30 mg/kg). Immunosuppressed mice were infected by nasal instillation with lethal doses either of *Klebsiella pneumoniae* IHMA1280740 co-producing SHV-OSBL (original-spectrum β-lactamase), TEM-OSBL, CTX-M-15 (ESBL), and DHA-1 (plasmid-encoded AmpC) β-lactamases, or of *Escherichia coli* NCTC13441 co-producing TEM-OSBL, CTX-M-15, and OXA-1/30 (class D) β-lactamases. Vehicle, cefepime, cefepime/AAI101 (2/1 $^{w}/_{w}$), or meropenem was administered IV q4h beginning 2 h post-infection. At 26 h post-infection lungs were excised and their pulmonary bioburdens determined by quantitative culture. Data were analyzed using the Kruskal-Wallis test, with the Conover-Inman *post-hoc* test for all pairwise comparisons between groups.

Results: Modal MICs (μg/mL) for the test strains were as follows:

Strain	Cefepime	Cefepime + 4 μg/mL AAI101	Meropenem
K. pneumoniae IHMA1280740	>128	0.06	0.125
E. coli NCTC13441	32	0.25	0.06

In plasma and lung epithelial lining fluid (ELF) the elimination half-life for cefepime was *ca.* 11 min, and for AAI101 *ca.* 14 min; lung penetration relative to plasma of cefepime and of AAI101 was *ca.* 36% and *ca.* 76%, respectively. *K. pneumoniae* IHMA1280740 and *E. coli* NCTC13441 each demonstrated robust post-infection growth in mouse lungs. A dose-response effect for cefepime/AAI101 was observed over the range 6.25/3.125-100/50 mg/kg for both test strains. For the same concentration of cefepime, cefepime/AAI101 combination treatment showed statistically significant superior efficacy to cefepime monotherapy ($P < 0.0001$) against both strains, and equivalent or superior efficacy to meropenem.

Conclusions: The experimental extended-spectrum β-lactamase inhibitor AAI101 restored efficacy to cefepime in a neutropenic murine model of pneumonia caused by cefepime-resistant Enterobacteriaceae co-producing ESBL and OSBL-type β-lactamases plus an AmpC or an OXA β-lactamase. AAI101 in combination with cefepime represents a promising new therapeutic modality for treatment of infections caused by drug-resistant Enterobacteriaceae.

INTRODUCTION

ESBL-producing Enterobacteriaceae recently were classified by the World Health Organization as “Priority Pathogen: CRITICAL”, and the spread of multidrug-resistant (MDR) Gram-negative bacteria continues at an unprecedented rate.

β-Lactam antibiotics have been the bedrock of antimicrobial therapy for over 70 years. The pharmaceutical industry kept pace with resistance by generating increasingly broader-spectrum and β-lactamase-resistant analogues. Additionally, older β-lactams have been rejuvenated by use in combination with β-lactamase inhibitors.

The objective of this study was to assess the potential of Allecra’s lead β-lactamase inhibitor AAI101 to restore cefepime efficacy in murine models of pneumonia due to resistant Enterobacteriaceae co-expressing ESBLs and other β-lactamases.

METHODS

Bacteria: The bacterial pathogens used in this study are listed in Table 1.

MICs: These were obtained, following CLSI guidelines, for cefepime alone, cefepime combined with fixed concentrations of 4 μg/mL and 8 μg/mL of AAI101, and meropenem.

Mouse strain: ICR mice (6 mice per dosing regimen per pathogen) were used.

PK study: Following single IV doses of cefepime/AAI101 60/30 mg/kg, blood and bronchoalveolar lavage (BAL) samples were collected over 8 h. Cefepime, AAI101, and urea concentrations were determined and PK parameters calculated.

Immunosuppression: Cyclophosphamide was administered intraperitonially at day -4 and at day -1, to induce neutropenia throughout the infection period.

Infection: Mice were rendered unconscious using ketamine and xylazine. Bacterial suspension was administered intranasally.

Treatment: IV, q4h, commenced 2 h post-infection. Groups included pre-treatment, vehicle, cefepime, cefepime/AAI101, and meropenem. Lungs were harvested at 2 h and 26 h post-infection, homogenized and the infecting pathogens cultured quantitatively.

RESULTS

Table 1. Modal MICs of cefepime, cefepime + AAI101, and meropenem (≥3 replicates).
Addition of AAI101 at 4 μg/mL and 8 μg/mL reduced the cefepime MIC by ≥128-fold.

Strain	β-Lactamase molecular summary	MIC (μg/mL) / test article			
		Cefepime	Cefepime + 4 μg/mL AAI101	Cefepime + 8 μg/mL AAI101	Meropenem
***E. coli* NCTC13441**	CTX-M-15, OXA-1/30, TEM-1	32	0.25	0.125	0.06
***K. pneumoniae* IHMA1280740**	SHV-OSBL, TEM-OSBL, CTX-M-15, DHA-1	>128	0.06	0.125	0.125

Table 2. Single-dose (IV) parameters for cefepime and AAI101 in mouse plasma & ELF.
Cefepime and AAI101 had similar half-lives and good penetration into ELF.

	Plasma Cefepime (60 mg/kg)	ELF Cefepime (60 mg/kg)	Plasma AAI101 (30 mg/kg)	ELF AAI101 (30 mg/kg)
Single Dose Parameters				
Elimination half-life (h)	0.191	0.186	0.224	0.242
Descriptive Curve Parameters				
$C_{initial}$ (ng/mL)	29408	10402	6774	4865
Curve area calculations				
$AUC_{0\text{-}3t}$ (ng·h/mL)	8397	2987	2197	1666
$AUC_{0\text{-}\infty}$ (ng·h/mL)	8609	3069	2309	1773

Table 3. Time point-specific ELF:plasma ratios for cefepime and AAI101 in mice.
High lung penetrations were achieved, with ratios of 0.4-0.6 (cefepime) and 0.7-0.9 (AAI101).

Time (h)	ELF Cefepime ng/mL	Plasma Cefepime ng/mL	Ratio ELF:Plasma Cefepime	ELF AAI101 ng/mL	Plasma AAI101 ng/mL	Ratio ELF:Plasma AAI101
0.083	8632	22905	0.38	4687	6507	0.72
0.25	4952	10844	0.46	2085	2730	0.76
0.5	2056	4956	0.41	978	1193	0.82
1	478	774	0.62	307	345	0.89

Figure 1. Tissue burden following treatment of *E. coli* NCTC13441 with cefepime/AAI101 is superior to cefepime monotherapy in a 26-h neutropenic murine pneumonia model.

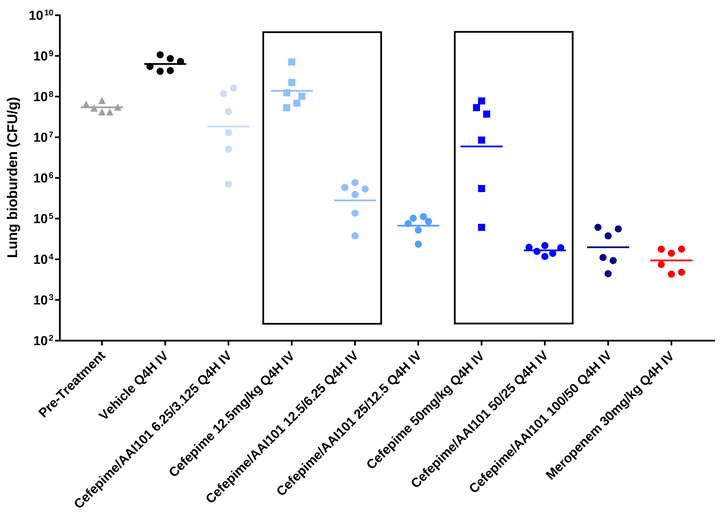

Figure 2. Tissue burden following treatment of *K. pneumoniae* IHMA1280740 with cefepime/AAI101 is superior to cefepime monotherapy in a 26-h neutropenic murine pneumonia model.

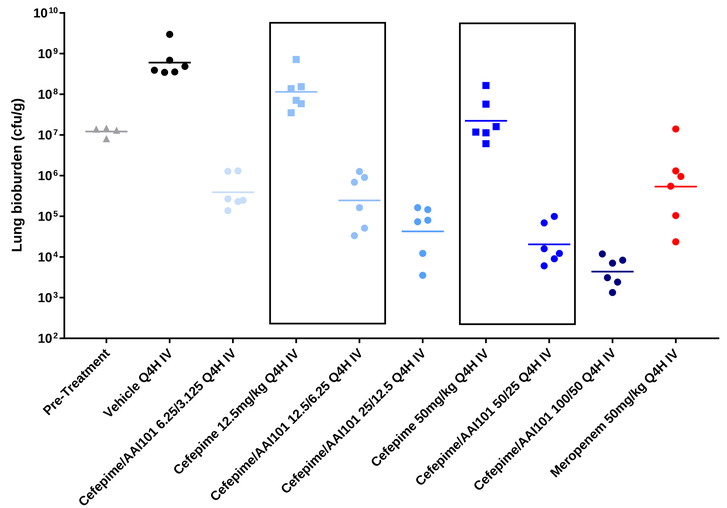

Table 4. Lung bioburdens following treatment of *E. coli* NCTC13441 or *K. pneumoniae* IHMA1280740 with cefepime/AAI101 are statistically significantly lower than those following cefepime monotherapy in a 26-h neutropenic murine pneumonia model. P-values compare drug treatment to vehicle.

Treatment	*E. coli* NCTC13441			*K. pneumoniae* IHMA1280740		
	log_{10} Group geometric mean (CFU/g)	log_{10} Reduction from pre-treatment (CFU/g)	P-value	log_{10} Group geometric mean (CFU/g)	log_{10} Reduction from pre-treatment (CFU/g)	P-value
Pre-treatment	7.74	-	-	7.08	-	0.0005
Vehicle IV Q4H	8.81	- 1.07	-	8.78	- 1.70	-
Cefepime 12.5 mg/kg IV Q4H	8.14	- 0.40	0.0355	8.06	- 0.98	0.1302
Cefepime 50 mg/kg IV Q4H	6.78	0.96	<0.0001	7.35	- 0.27	0.0008
Cefepime/AAI101 6.25/3.125 mg/kg IV Q4H	7.26	0.48	<0.0001	5.59	1.49	<0.0001
Cefepime/AAI101 12.5/6.25 mg/kg IV Q4H	5.45	2.29	<0.0001	5.39	1.69	<0.0001
Cefepime/AAI101 25/12.5 mg/kg IV Q4H	4.83	2.91	<0.0001	4.63	2.45	<0.0001
Cefepime/AAI101 50/25 mg/kg IV Q4H	4.22	3.52	<0.0001	4.31	2.77	<0.0001
Cefepime/AAI101 100/50 mg/kg IV Q4H	4.30	3.44	<0.0001	3.64	3.44	<0.0001
Meropenem IV Q4H*	3.97	3.76	<0.0001	5.73	1.35	<0.0001

*Meropenem dosed IV at 30 mg/kg for *E. coli*, 50 mg/kg for *K. pneumoniae*.

CONCLUSIONS

- Cefepime/AAI101 proved highly efficacious at reducing lung bioburdens in the neutropenic murine pneumonia model.
- AAI101 restored the efficacy of cefepime against ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* also co-producing other β-lactamase classes.
- Efficacy of cefepime/AAI101 was achieved using clinically feasible cefepime/AAI101 treatment regimens. AAI101 achieved excellent penetration into lung ELF (70-90% of plasma levels).
- These studies support continued clinical development of cefepime combined with AAI101 to treat infections caused by MDR Enterobacteriaceae expressing extended-spectrum β-lactamases.