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Comparative Efficacy of Human Simulated Exposures of Cefepime and Cefepime/AAI101 against Clinical Multidrug-Resistant Enterobacteriaceae

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ABSTRACT (revised)

Background: The combination of cefepime (FEP) with the novel extended-spectrum beta-lactamase inhibitor AAI101 possesses potent *in vitro* activity against many resistant Gram-negative pathogens. We evaluated the *in vivo* efficacy of human simulated doses of FEP alone and FEP/AAI101 using the neutropenic murine thigh infection model.
Methods: Twenty clinical MDR Enterobacteriaceae (3 *Escherichia coli* and 17 *Klebsiella pneumoniae)* with FEP MICs of 8 to >64 mg/L and FEP/AAI101 MICs (fixed AAI101 concentration of 8 mg/L) of 2 to >64 mg/L were utilized. Two hours after inoculation mice were dosed with regimens simulating the free drug concentration-time profile seen in man given 2g every 8 h (30-min infusion) of FEP, with or without AAI101 at doses of 2g or 0.5g every 8 h (30-min infusion). Efficacy was calculated as the change in thigh bacterial density (log_{10} CFU) after 24 h relative to the starting inoculum (0 h).
Results: Bacterial growth (mean ± SE log_{10} CFU) of 2.7 ± 0.1 was observed in untreated animals after 24 h. For FEP monotherapy efficacy (-0.9 ± 0.2) was observed against 3 isolates with FEP MICs of 8, 32, and >64 mg/L, whereas increases in bacterial density similar to control animals (1.9 ± 0.1) were noted for the remaining 17 strains with FEP MICs ≥64 mg/L. FEP/2g AAI101 treatment resulted in bacterial reductions for 19 of the 20 strains tested: -1.0 ± 0.5, -1.4 ± 0.4, -1.1 ± 0.5, and -0.6 ± 0.5 for isolates with FEP/AAI101 MICs of ≤16 mg/L (FEP *f*T>MIC ≥ 65%), 32 mg/L (FEP *f*T>MIC = 38%), 64 mg/L (FEP *f*T>MIC = 20%), and >64 mg/L (FEP *f*T>MIC = 1%), respectively. FEP/0.5g AAI101 treatment resulted in bacterial reductions in 15 of the 16 strains with FEP/AAI101 MICs ≤32 mg/L: -1.1 ± 0.1, -0.6 ± 0.1, -0.5 ± 0.1, and -0.7 ± 0.3 for isolates with FEP/AAI101 MICs (mg/L) of ≤4, 8, 16, and 32, respectively.
Conclusions: FEP monotherapy resulted in minimal *in vivo* activity against this highly FEP-resistant bacterial population. Conversely, FEP/AAI101 administered using a regimen simulating human exposures of 2 g/2 g or 2 g/0.5 g q8h resulted in activity against most of these MDR Enterobacteriaceae. These data support the potential utility of FEP/AAI101 for treatment of resistant Gram-negative pathogens.

INTRODUCTION

- Production of β-lactamases by Gram-negative bacteria compromises the clinical value of diverse β-lactams, including penicillins, cephalosporins, and carbapenems.
- Considerable success has been achieved by combining β-lactam antibiotics with β-lactamase inhibitors, notable examples being piperacillin/tazobactam, amoxicillin/clavulanate, and ampicillin/sulbactam.
- AAI101, an extended-spectrum β-lactamase inhibitor,[1,2] currently is in clinical development in combination with the commonly utilized cephalosporin, cefepime.
- Using the neutropenic murine thigh infection model, we sought to evaluate human simulated doses of cefepime/AAI101 combinations against multidrug-resistant Enterobacteriaceae and to compare these activities to that of cefepime alone.

METHODS

Antimicrobial test agents

- Commercially available cefepime (Sagent Pharmaceuticals, Schaumburg, IL, USA) was obtained from the Hartford Hospital Pharmacy Department, and AAI101 was supplied by Allecra Therapeutics SAS (St-Louis, France).

Bacterial isolates and* in vitro *susceptibility

- Twenty MDR Gram-negative clinical isolates (3 *E. coli*, 17 *K. pneumoniae*) were recruited for these experiments. Strains were collected from a large surveillance study of hospitals across the USA in 2013-2014.
- MICs for cefepime and cefepime/AAI101 were determined by broth microdilution, using breakpoint assignments prescribed by the Clinical and Laboratory Standards Institute.[3] For cefepime/AAI101 doubling dilutions of cefepime were utilized in combination with a fixed 8 mg/L concentration of AAI101. MICs were obtained at least in triplicate, and the median MIC is reported.

Neutropenic thigh infection model

- This study was reviewed and approved by the Hartford Hospital Institutional Animal Care and Use Committee.
- Female ICR mice (Harlan Sprague Dawley, Inc., Indianapolis, IN), each weighing *ca.* 25 g, were used throughout the study.
- Mice were rendered transiently neutropenic by intraperitoneal (IP) injections of cyclophosphamide 150 and 100 mg/kg at 4 days and 1 day, respectively, prior to inoculation.
- Three days prior to inoculation, mice also received a single 5 mg/kg IP injection of uranyl nitrate to slow drug clearance.
- Two h prior to initiation of antimicrobial therapy, left and right thighs of each mouse were inoculated intramuscularly with 0.1 mL containing *ca.* 10^7 CFU/mL of test isolate suspended in physiological saline.
- This neutropenic murine thigh infection model was applied to all pharmacokinetic (PK) and pharmacodynamic experiments.

Human simulated dosing regimen determination

- Single-dose PK studies of cefepime (as previously determined by our group[4]) and of cefepime/AAI101 were undertaken in infected neutropenic animals, and mean PK parameters determined (WinNonlin Version 5.0.1; Pharsight, Mountain View, CA). Using these parameters, we simulated median values for the *f*T>MIC observed in healthy human volunteers given 2g cefepime alone every 8 h as a 30-min infusion as monotherapy, or 2 g cefepime combined with AAI101 at a dose of 2g or 0.5g every 8 hours as a 30-min infusion (data on file, Allecra Therapeutics SAS).
  - Mice received single doses of the compounds, and groups of six mice were euthanized at eight time points throughout the 8-h dosing interval.
  - Blood samples were taken *via* cardiac puncture, and serum was stored at -80 ºC until analysis.
  - Cefepime concentrations were quantified at the Center for Anti-Infective Research and Development (Hartford, CT) by HPLC,[5] whereas AAI101 was quantified at Aptuit S.r.l. (Verona, Italy) using a validated LC-MS-MS protocol.
- Confirmatory PK studies were undertaken in infected mice to confirm target exposures prior to use of these regimens in pharmacodynamic analyses.
  - Infected neutropenic mice were dosed with the calculated regimens, and groups of 6 mice were euthanized at 4 to 6 time points throughout the 8-h dosing interval to confirm target exposures.

Protein Binding Studies

- Cefepime protein binding values of 0% for mice[6] and 20% for man[7] were utilized.
- AAI101 protein binding in mouse and human serum was determined over a range of concentrations.
  - Studies were conducted as three independent tests using Amicon Centrifree® Micropartition devices (Millipore, Bedford, MA) with 30,000 MW cut-off filters, according to the manufacturer's instructions.
  - Solutions were made in freshly collected human and mouse serum, heated at 37 ºC in a shaking water bath for 10 min, then centrifuged for 45 min at 10 ºC at 2000 × *g*.
  - AAI101 concentrations of 200, 50, and 5 mg/L were evaluated, as was non-specific binding to the filter device at an AAI101 concentration of 50 mg/L.

Efficacy as assessed by bacterial density

- For each of the 20 Gram-negative isolates, groups of three neutropenic mice received cefepime or cefepime/AAI101 human simulated regimens beginning 2 h post-infection (0 h).
- All doses were administered as 0.2 mL subcutaneous injections, and consisted of three 8-h dosing intervals over 24 h.
- Control animals received physiological saline using the same volume, route, and frequency as treatment regimens.
- Control mice (three per group) were sacrificed just prior to antibiotic initiation (0 h) and at 24 h after treatment initiation, whereas treatment mice (three per group) were sacrificed 24 h after start of treatment.
- After sacrifice individual thighs were homogenized in physiological saline and plated on Trypticase soy agar with 5% sheep blood (BD, Franklin Lakes, NJ) for CFU determination.
- Efficacy was calculated as the change in bacterial density (Δ log_{10}CFU) after 24 h as compared with 0-h control animals.

RESULTS

In vitro susceptibility

- The phenotypic profiles for the 20 isolates used in efficacy studies are shown in **Table 1**.

Protein Binding Studies

- AAI101 was not bound appreciably to proteins in either human or ICR mouse plasma,so, AI101 concentrations were assumed to be 100% free.

Dosing regimen determination

- The PK of both cefepime and AAI101 were best described using a 1-compartment model with first-order input and elimination.
- Human simulated regimens consisted of 2 doses for each 8-h dosing interval. *In vivo* free drug PK profiles were as follows:
  - 2g cefepime (**Figure 1**), 2g cefepime/2g AAI101 (**Figure 2**), 2g cefepime/0.5g AAI101 (**Figure 3**)
- The cefepime *f*T>MIC and AAI101 *f*T>concentration values attained for these regimens in mice, and those anticipated in man, highlight the similarities between exposures (**Table 2A and 2B**).

Efficacy as assessed by changes in bacterial density

- The results of the efficacy experiments for 2g cefepime/2g AAI101 are shown in **Figure 4,** and those for 2g cefepime/0.5g AAI101 in **Figure 5**.
  - Consistent with elevated MICs, cefepime monotherapy reduced bacterial density against only 3 of 20 isolates evaluated (cefepime MICs of 8, 32, and >64 µg/mL), with increases in bacterial density similar to saline controls for the remaining 17 strains.
  - Treatment with 2g cefepime/2g AAI101 resulted in bacterial load reductions of ≥0.5 log_{10} CFU for 18 of 20 strains tested, whereas treatment with 2g cefepime/0.5 g AAI101 did so in 12 of 20 isolates.
  - The two dosing regimens resulted in similar bacterial load reduction for all strains with cefepime/AAI101 MICs ≤4 mg/L, which represent 80% of the overall Enterobacteriaceae distribution.[8]
  - Increases in bacterial density with 2g cefepime/0.5 g AAI101 was only seen with 4 strains (all with cefepime/AAI101 MIC ≥32 µg/mL)..

Table 1. Minimum inhibitory concentration (mg/L) profile of the 20 Enterobacteriaceae isolates utilized in efficacy studies.

Isolate	FEP	FEP/AAI	TZP	MEM	CIP	TOB
KP C13-10	64	2	>256	16	>16	32
KP C16-9	>64	2	>256	1	>16	16
KP C22-6	8	4	>256	0.25	4	0.5
EC C22-30	32	4	>256	16	>64	16
EC C16-4	>64	8	>256	16	1	64
KP C31-2	>64	8	>256	32	>16	>64
KP C31-14	>64	8	>256	64	>16	0.5
KP C37-25	>64	8	>256	32	>16	32
KP C4-25	64	16	>256	64	>16	16
KP C37-28	>64	16	>256	16	>16	16
KP C21-22	>64	16	>256	32	>64	32
KP C31-18	>64	16	>256	32	>16	16
KP C6-5	>64	32	>256	32	>16	32
KP C13-25	>64	32	256	>64	2	16
KP C19-1	>64	32	>256	16	>64	32
KP C30-5	>64	32	>256	64	>16	32
KP C30-27	>64	64	>256	>64	>16	32
EC C3-14	>64	64	>256	4	4	64
KP C4-10	>64	>64	>256	>64	>16	32
KP C8-9	>64	>64	>256	>64	>16	32

KP, *K. pneumoniae;* EC, *E. coli;* FEP, cefepime; FEP/AAI, cefepime/AAI101; TZP, piperacillin/tazobactam; MEM, meropenem; CIP, ciprofloxacin; TOB, tobramycin

Figure 1. Free concentration-time profiles for the human simulated regimen of cefepime 2g every 8 hours (30-min infusions) in mice as compared with humans. Symbols represent mean ± SD.

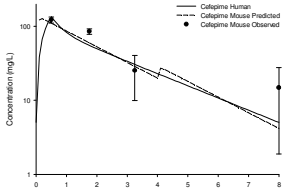


Figure 2. Free concentration-time profiles for the human simulated regimen of 2g cefepime/2g AAI101 every 8 hours (30-min infusions) in mice as compared with humans. Symbols represent mean ± SD.

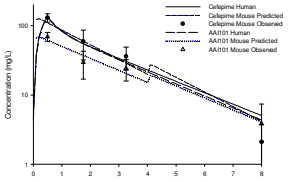


Figure 3. Free concentration-time profiles for the human simulated regimen of 2g cefepime/0.5g AAI101 every 8 hours (30-min infusions) in mice as compared with humans. Symbols represent mean ± SD.

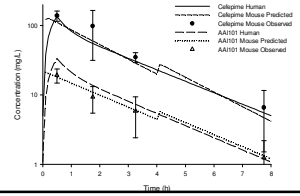


Table 2. Human simulated *f*T>MIC profile for (A) cefepime 2g every 8 hours (30-min infusion) and (B) AAI101 2g or 0.5g every 8 hours (30-min infusions) in man and in mice. AAI101 coadministration had negligible impact on the cefepime profile.

(A)

MIC (mg/L)	Cefepime *f*T>MIC (%) Human	Mouse
4	100	100
8	84	83
16	61	65
32	39	38
64	16	20
128	1	1

(B)

Concentration (mg/L)	AAI101 *f*T>concn (%) 2g Human	2g Mouse	0.5g Human	0.5g Mouse
1	100	100	100	100
2	100	100	80	85
4	100	100	59	63
8	80	80	37	33
16	59	57	16	11
32	37	28	1	0
64	16	6	0	0
128	1	0	0	0

Figure 4. Comparative efficacies of humanized 2g cefepime/2g AAI101 (FEP/AAI) and cefepime alone against a distribution of Enterobacteriaceae. Bars represent mean ± SD.

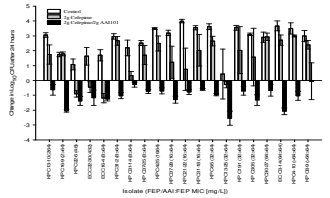
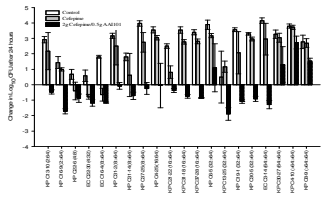


Figure 5. Comparative efficacies of humanized 2g cefepime/0.5g AAI101 (FEP/AAI) and cefepime alone against a distribution of Enterobacteriaceae. Bars represent mean ± SD.



CONCLUSIONS

- Addition of AAI101 to cefepime resulted in large shifts in MIC, highlighting the role β-lactamases play in resistance to these pathogens.
- Given the high rates of cefepime non-susceptibility for the chosen strains, monotherapy with high-dose cefepime resulted in minimal activity against these MDR organisms.
- Addition of AAI101 dosed at 2g every 8 hours restored cefepime *in vivo* activity against all but organisms with the highest cefepime/AAI101 MICs (*i.e.* >64 mg/L).
- Activity of cefepime when combined with 0.5g of AAI101 resulted in bacterial reductions against most strains with cefepime/AAI101 MICs ≤32 mg/L.
- These data support continued development of cefepime/AAI101 for treatment of infections attributed to multidrug-resistant Enterobacteriaceae.

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