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Supporting the ceftaroline fosamil/avibactam Enterobacteriaceae breakpoint determination using humanised in vivo exposures in a thigh model

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ABSTRACT

Previous in vivo studies using a human-simulated regimen of ceftaroline/avibactam 600/600 mg every 8 h (q8h) showed activity against extended-spectrum β-lactamase-, AmpC- and KPC-producing Enterobacteriaceae with minimum inhibitory concentrations (MICs) ≤1 μg/mL. Here we sought to determine the efficacy of this human-simulated regimen against organisms with MICs ≥1 μg/mL to help determine a breakpoint value that would reliability predict efficacy in humans. In total, 31 isolates (1 *Escherichia coli*, 9 *Klebsiella pneumoniae*, 9 *Enterobacter cloacae*, 1 *Citrobacter koseri*, 2 *Serratia marcescens*, 1 *Klebsiella oxytoca* and 8 *Pseudomonas aeruginosa*) with ceftaroline/avibactam MICs of 1 to 16 μg/mL were tested in a murine immunocompromised thigh infection model; 15 isolates were also tested in an immunocompetent model. Doses were given to simulate human free drug exposures of ceftaroline fosamil/avibactam 600/600 mg q8h over 24 h as a 1-h infusion by targeting the $fT_{>MIC}$ profile. Efficacy was evaluated as the change in $\log_{10}$ CFU compared with 0-h controls after 24 h. Reductions in bacterial CFU in the neutropenic model were seen against a majority of isolates tested with MICs ≤4 μg/mL, where $fT_{>MIC}$ was >55%. More variable efficacy was seen in isolates with MICs ≥8 μg/mL, where $fT_{>MIC}$ drops below 40%. Overall activity was enhanced in the immunocompetent model. The humanised regimen of ceftaroline fosamil/avibactam 600/600 mg q8h as a 1-h infusion showed predictable efficacy against isolates with various genotypic and phenotypic profiles and MICs ≤4 μg/mL. These data provide valuable information to help determine a ceftaroline/avibactam breakpoint for Enterobacteriaceae.

1. Introduction

Clinical breakpoints are essential to help determine when a given antimicrobial agent is appropriate in treating a pathogen. They help guide clinicians in selecting appropriate antimicrobial therapy to ensure successful outcomes and to minimise the emergence of resistance. In vivo animal models have been used extensively to help define the pharmacokinetic/pharmacodynamic (PK/PD) relationships of many classes of antimicrobials such as aminoglycosides, β-lactams and fluoroquinolones [1]. These models are often utilised to generate data for novel compounds and to define the PK/PD targets required for efficacy. In addition, to fully understand and assess the pharmacodynamic profile of an agent, it is necessary to define targets for both efficacy and failure, which are not ethical to assess in clinical studies. Use of humanised regimens in animal models, based on pharmacodynamic targets in man, provide considerable clinical utility as they assess the robustness of activity against a range of phenotypically and genotypically diverse isolates. Given the increase in resistant Enterobacteriaceae, defining an appropriate breakpoint for novel compounds with activity against these organisms is especially valuable.

Enterobacteriaceae are often responsible for a variety of severe infections such as sepsis, complicated urinary tract infections and pneumonia [2]. Specifically, extended-spectrum β-lactamases (ESBLs) and carbapenemases, such as *Klebsiella pneumoniae* carbapenemases (KPCs), are of particular concern due to their increasing prevalence and multidrug resistance profiles that diminish the clinical utility of many currently available antimicrobial treatment options [3,4].

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Ceftaroline fosamil, a cephalosporin with activity against meticillin-resistant *Staphylococcus aureus* (MRSA), has considerable activity against many common Gram-positive and Gram-negative organisms, including Enterobacteriaceae [5]. However, in the presence of β-lactamases, such as KPCs and ESBLs, the activity of this compound is severely decreased [4,6,7]. Whilst avibactam, a novel non-β-lactam β-lactamase inhibitor, has no inherent antibacterial activity, it has the ability to prevent the hydrolysis of β-lactams and therefore restores the potency of these antibacterials against organisms producing enzymes such as AmpC, ESBL and KPC [4,8,9]. Previously, ceftaroline/avibactam has been shown to be effective in vivo against ESBL- and non-ESBL-producing Enterobacteriaceae with minimum inhibitory concentrations (MICs) ≤1 μg/mL [10]. In the current study, the efficacy of a humanised exposure of ceftaroline/avibactam 600/600 mg every 8 h (q8h) as a 1-h infusion was tested in neutropenic and immunocompetent murine thigh infection models against Gram-negative isolates with various resistance mechanisms exhibiting MICs ≥1 μg/mL.

2. Materials and methods

2.1. Antimicrobial test agents

Commercially available ceftaroline fosamil (Lot no. 0002D16) was obtained from the Hartford Hospital Pharmacy Department (Hartford, CT), and analytical grade avibactam (Lot no. AFCH005151; Forest Laboratories, Inc., New York, NY) was used for all in vivo analyses. Clinical vials of ceftaroline fosamil were reconstituted as described in the prescribing information and were diluted as appropriate to achieve the desired concentrations; analytical avibactam powder was weighed in a quantity sufficient to achieve the required concentrations and was reconstituted immediately prior to each in vivo experiment. The combined ceftaroline and avibactam solutions were stored under refrigeration and were discarded within 24 h of reconstitution.

2.2. Bacterial isolates

A total of 31 isolates (1 *Escherichia coli*, 9 *K. pneumoniae*, 9 *Enterobacter cloacae*, 1 *Citrobacter koseri*, 2 *Serratia marcescens*, 1 *Klebsiella oxytoca* and 8 *Pseudomonas aeruginosa*) were obtained from JMI Laboratories (North Liberty, IA) and were used for the in vivo studies. MICs were determined using the standard broth microdilution technique as outlined by the Clinical and Laboratory Standards Institute (CLSI) with doubling dilutions of ceftaroline and a fixed avibactam concentration of 4 μg/mL. All isolates were characterised using a gene microarray system manufactured by Checkpoints and the Check-Points Check-MDR CT103 Kit (Check-Points Health B.V., Wageningen, The Netherlands). Although this agent is not intended for antipseudomonal use, the highest ceftaroline/avibactam MIC against an Enterobacteriaceae that we were able to acquire was 8 μg/mL and therefore eight *P. aeruginosa* isolates were included to extend the phenotypic profile up to 16 μg/mL. Isolates were maintained in double-strength skim milk (BD Biosciences, Sparks, MD) at −80 °C. Each isolate was subcultured twice on trypticase soy agar with 5% sheep blood (BD Biosciences) prior to use in the experiments.

2.3. Neutropenic thigh infection model

Prior to initiation of the animal studies, the protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Hartford Hospital. Pathogen-free, female ICR mice weighing ca. 20–22 g were acquired from Harlan Sprague Dawley, Inc. (Indianapolis, IN) and were utilised throughout these experiments. Animals were maintained and used in accordance with National Research Council recommendations and were provided food and water ad libitum. Mice were rendered neutropenic with 100 mg/kg and 150 mg/kg intraperitoneal injections of cyclophosphamide (Cytoxan®; Bristol-Myers Squibb, Princeton, NJ) given 1 day and 4 days prior to inoculation, respectively. Three days prior to inoculation, mice were also given a single 5 mg/kg intraperitoneal injection of uranyl nitrate. This produces a predictable degree of renal impairment to slow drug clearance [11]. Two hours prior to initiation of antimicrobial therapy, each thigh was inoculated intramuscularly with a 0.1 mL solution containing 10^7 CFU of the test isolate.

2.4. Immunocompetent thigh infection model

Mice utilised in the immunocompetent model underwent the same procedure as the neutropenic mice with the exception of the administration of cyclophosphamide. In addition, a final inoculum of 10^8 CFU was used to produce thigh infection. A higher inoculum was utilised for the immunocompetent model to counteract the presence of the immune system and to ensure adequate development of infection.

2.5. Determination of dosing regimen

A humanised dosing regimen of ceftaroline fosamil 600 mg and avibactam 600 mg q8h that was previously developed and validated in infected neutropenic and immunocompetent models by our group was utilised in the current investigations [10]. Briefly, single-dose pharmacokinetic studies were conducted at doses of 12.5, 25 and 50 mg/kg. Pharmacokinetic parameters from these single-dose studies were calculated and were used to determine the humanised regimen. The humanised regimen was designed in mice to simulate the time that free drug concentration remains above the MIC ($fT_{>MIC}$), the peak concentration (C_{max}) and the area under the concentration–time curve (AUC) observed in humans following administration of ceftaroline/avibactam 600/600 mg q8h as a 1-h infusion. The dosing regimen administered over the 8-h interval was 25 mg/kg at 0 h, followed by 3.125 mg/kg at 2, 3.5 and 6 h. This regimen was repeated every 8 h for a 24-h period. The $fT_{>MIC}$ exposure profiles of both ceftaroline and avibactam were shown to be similar between humans and mice, as displayed in Table 1 [10].

2.6. In vivo efficacy

A total of 31 isolates were used in the efficacy studies, with MICs ranging from 1 μg/mL to 16 μg/mL; 15 of these isolates were also utilised in the immunocompetent studies. Beginning 2 h after inoculation, groups of three mice were administered the human-simulated regimen of ceftaroline fosamil and avibactam over a 24-h period. All doses were administered as 0.2-mL subcutaneous injections. Control animals were administered normal saline at the same volume, route and frequency as the treatment regimen. Groups of three untreated control mice were euthanised by CO_2 exposure, followed by cervical dislocation just prior to initiation of therapy

Table 1
$fT_{>MIC}$ values achieved for ceftaroline with a human-simulated regimen of ceftaroline/avibactam 600/600 mg every 8 h in mice and corresponding targets in humans.

	MIC (μg/mL)					
	≤1	2	4	8	16	32
$fT_{>MIC}$ (mouse)	100	86	56	33	14	0
$fT_{>MIC}$ (human)	100	85	55	29	9	0

MIC, minimum inhibitory concentration; $fT_{>MIC}$, time that free drug concentration remains above the MIC.