Ceftaroline plus Avibactam Demonstrates Bactericidal Activity against Pathogenic Anaerobic Bacteria in a One-Compartment In Vitro Pharmacokinetic/Pharmacodynamic Model

Brian J. Werth, Michael J. Rybak

Anti-Infective Research Laboratory, Eugene Applebaum College of Pharmacy and Health Sciences, and School of Medicine, Wayne State University, Detroit, Michigan, USA

Anaerobic pathogens are often associated with polymicrobial infections, such as diabetic foot infections. Patients with these infections are often treated with broad-spectrum, multidrug therapies targeting resistant Gram-positive bacteria, such as methicillin-resistant Staphylococcus aureus, as well as Gram-negative bacteria and anaerobes. The broad-spectrum, non-beta-lactam, beta-lactamase inhibitor avibactam has been combined with ceftaroline and may provide a single-product alternative for complicated polymicrobial infections. We compared the activity of ceftaroline-avibactam (CPA) to that of ertapenem (ERT) against common anaerobic pathogens in an in vitro pharmacokinetic/pharmacodynamic (PK/PD) model. Simulations of doses of ceftaroline-fosamil at 600 mg every 8 h (q8h) (maximum free drug concentration \(f_{C_{\text{max}}}\), 17.04 mg/liter, and half-life \(t_{1/2}\), 2.66 h) plus avibactam at 600 mg q8h \(f_{C_{\text{max}}}\), 11.72 mg/liter, and \(t_{1/2}\), 1.8 h) and of ertapenem at 1 g q24h \(f_{C_{\text{max}}}\), 13 mg/liter, and \(t_{1/2}\), 4 h) were evaluated against two strains of Bacteroides fragilis, one strain of Prevotella bivia, and one strain of Finegoldia magna in an anaerobic one-compartment in vitro PK/PD model over 72 h with a starting inoculum of \(\approx 8 \log_{10} \text{CFU/ml}\). Bactericidal activity was defined as a reduction of \(\geq 3 \log_{10} \text{CFU/ml}\) from the starting inoculum. Both CPA and ERT were bactericidal against all four strains. CPA demonstrated improved activity against Bacteroides strains compared to that of ERT but had similar activity against Finegoldia magna and P. bivia, although modest regrowth was observed with CPA against P. bivia. No resistance emerged from any of the models. The pharmacokinetics achieved were 92 to 105% of the targets. CPA has potent in vitro activity against common anaerobic pathogens at clinically relevant drug exposures and may be a suitable single product for the management of complicated polymicrobial infections.

Anaerobic bacteria constitute the majority of normal skin and mucous membrane flora in humans, reaching the highest densities in the alimentary canal and female reproductive tract (1–3). Under normal circumstances, these organisms play a symbiotic role, aiding in metabolic functions and preventing colonization and invasion of pathogens at these sites (2, 4). However, when this equilibrium is disrupted, commensal organisms can become pathogenic through virulence factor production and breakdown of membranes that protect sterile sites. These infections tend to be polymicrobial and can be complicated by bacterial factors like oxygen tolerance, drug resistance, and virulence factor production, host factors like impaired immune function, and diagnostic challenges like low rate of recovery of anaerobic pathogens in culture, as well as an inability to distinguish pathogenic and commensal strains from infected sites (5). Anaerobic bacteria can be associated with a multitude of infections at many anatomical sites but are frequently implicated in diabetic foot infections (DFI) (6–8). While aerobic Gram-positive cocci, such as Staphylococcus aureus, are the most common pathogens isolated from DFI, aerobic Gram-negative bacteria and obligate anaerobes, such as peptostreptococci, Finegoldia magna, and Bacteroides fragilis, are often present in severe or chronic DFI (6–9).

Cefaroline-fosamil is a broad-spectrum cephalosporin with activity against typical bacterial pathogens, including methicillin-resistant Staphylococcus aureus (MRSA), penicillin-resistant streptococci, and many aerobic Gram-negative organisms (10). The addition of the novel, non-beta-lactam, beta-lactamase inhibitor avibactam (formerly NXL-104) to ceftaroline (ceftaroline-avibactam [CPA]) further extends this coverage to include extended-spectrum-beta-lactamase (ESBL)- and Klebsiella pneumoniae carbapenemase (KPC)-producing enterobacteriaceae, as well as anaerobes (7, 11–13). A recent study evaluating the activity of this combination against 470 pathogens isolated from DFIs, including 154 anaerobes, demonstrated that ceftaroline plus avibactam had broader coverage against the collection of strains than any of the comparator agents (7). Due to the broad coverage of common DFI pathogens, this combination offers a single-product option for treatment of polymicrobial infections like complicated skin and soft tissue infections associated with peripheral vascular insufficiency, as seen in DFI.

The purpose of the present study was to quantify and compare the antibacterial activities of CPA and ertapenem (ERT) against common anaerobic pathogens in an in vitro pharmacokinetic/pharmacodynamic (PK/PD) model simulating average human pharmacokinetic exposures.

MATERIALS AND METHODS

Bacterial strains. Four strains with previously determined MICs were selected from the collection at Forest Laboratories and evaluated in the...
PK/PD model. One clinical strain each of *Bacteroides fragilis*, *Prevotella bivia*, and *F. magna* was evaluated. These organisms and their susceptibility profiles are listed in Table 1.

**Antimicrobials.** Analytical grade cefaroline (lot number FMD-CEF-051) and avibactam (lot number L003145) powders were provided by Forest Laboratories (New York, NY). Ertapenem (lot number 2055490) was purchased commercially (Merck & Co., Inc., Whitehouse Station, NJ).

**Media.** Wilkins-Chalgren broth (WCB; Oxoid, United Kingdom) was used for all *in vitro* experiments. Colony counts were determined using Wilkins-Chalgren agar (WCA; Oxoid) plates.

**In vitro PK/PD model.** An *in vitro*, 1-compartment PK/PD model with a 250-ml capacity and input and outflow ports was used. Two 8-liter poly-methyl-methacrylate chambers with removable airtight lids were fabricated and used to maintain an anaerobic environment around the models. Each model was prefilled with medium and connected into the anaerobe chamber. Five anaerobic gas sachets (Pack-Anaero, Mistubishi Gas Chemical Company, Inc., Japan) were placed in the anaerobic chamber to remove oxygen from the system, and the lid was sealed. The models were maintained under anaerobic conditions at all times, which was confirmed with anaerobic indicator strips (Oxoid). Sampling ports were Y-sited into the outflow tubing to allow for sampling without disrupting the anaerobic conditions in the model chamber. The time between sampling, diluting, and plating the samples was minimized to limit oxygen exposure. Prior to each experiment, bacterial lawns from overdiluted inocula were washed through a 0.45-m filter with normal saline to remove the antimicrobial agent and capture surviving cells on the filter membrane. Colony counts were expressed as mean log$_{10}$ CFU/ml. For both methods, plates were incubated at 37°C for 48 h under anaerobic conditions, at which time colony counts were performed. These methods have a lower limit of reliable detection of 1 log$_{10}$ CFU/ml. The total change in log$_{10}$ CFU/ml was plotted against time to construct curves summarizing the pharmacodynamic activity of each pharmacokinetic exposure against each organism. Bactericidal activity (99.9% kill) was defined as a $\geq$3-log$_{10}$ CFU/ml decrease in colony count from the initial inoculum. Bacteriostatic activity was defined as a $<$3-log$_{10}$ CFU/ml reduction in colony count from the initial inoculum, while inactivity was defined as growth from the initial inoculum.

**Pharmacokinetic analysis.** Pharmacokinetic samples were drawn from the outflow port of each model at multiple time points to confirm the achievement of target antibiotic concentrations. All samples were stored at $\sim$70°C until ready for analysis. Cefaroline and avibactam concentrations were determined by a previously described liquid chromatography and mass spectrometry methodology (11). Ertapenem concentrations were determined by a previously described bioassay methodology using *Escherichia coli* NCTC 10418. Blank 1/4-in disks were spotted with 10 μl of the standards or samples. Each standard and sample was tested in duplicate. The standard concentrations ranged from 1 to 16 mg/liter (16).

The half-lives, peak concentrations, and time above the MIC ($T > MIC$) of the antibiotics were determined utilizing PK Analyst software (version 1.10; Micromath Scientific Software, Salt Lake City, UT).

**Resistance.** The development of resistance was evaluated at 24, 48, and 72 h by plating 100-μl samples on WCA plates supplemented with avibactam at 4 mg/liter and ceftaroline at a concentration of 3× MIC or ertapenem at a concentration of 3× MIC. Plates were then examined for growth after 48 h of incubation at 37°C under anaerobic conditions.

**Statistical analysis.** Changes in CFU/ml at 0, 4, 8, 24, 32, 48, 56, and 72 h were compared by one-way analysis of variance with Tukey's post hoc test. A P value of $\leq0.05$ was considered significant. All statistical analyses were performed using SPSS Statistical Software (release 21.0; SPSS, Inc., Chicago, IL).

**RESULTS**

**Susceptibilities.** All strains tested were susceptible to CPA and ertapenem. The MICs are listed in Table 1.

**In vitro PK/PD model.** The pharmacokinetic parameters achieved in the model were within 7.5% of the targeted parameters for ertapenem, with an intraday coefficient of variance (CV) of $<$5.4% for all standards tested. The cefaroline and avibactam concentrations were within 7.6% of targeted parameters, with a within-run CV of $\leq$3.2%. For ertapenem simulations, the $f_{\text{Cmax}}$ was 12.6 ± 2.15 μg/ml (mean $\pm$ standard deviation; target, 13 μg/ml), the $f_{\text{Cmin}}$ was 0.14 $\pm$ 0.01 μg/ml, and the $t_{1/2}$ was 3.71 $\pm$ 0.18 h (target 4 h). The time above the MIC ($T > MIC$) was 41.1% for the *B. fragilis* clinical strain (MIC of 2 μg/ml), 87.4% for ATCC 25285 (MIC of 0.25 μg/ml), 56.5% for *F. magna* (MIC of 1 μg/ml), and 87.4% for *P. bivia* (MIC of 0.25 μg/ml). The $f_{\text{Cmax}}$, $f_{\text{Cmin}}$, and $t_{1/2}$ were 15.7 $\pm$ 0.08 μg/ml (target, 17.04 μg/ml), 1.84 $\pm$ 0.06 μg/ml, and 2.66 $\pm$ 0.01 h (target, 2.66 h) for cefaroline and 11.97 $\pm$ 0.04 μg/ml (target, 11.72 μg/ml), 0.68 $\pm$ 0.12 μg/ml, and 1.93 $\pm$ 0.12 h (1.8 h) for avibactam. The $T > MIC$ for this combination was 98.8% for the *B. fragilis* clinical strain and 100% for *F. magna*, *B. fragilis* ATCC 25285, and *P. bivia*. The pharmacodynamic data at 72 h are summarized in Table 2, and changes in log$_{10}$ the concentration of antimicrobial remaining in the sample was anticipated to be near the MIC of the test organism, vacuum filtration was used to avoid antibiotic carryover. When vacuum filtration was used, samples were washed through a 0.45-μm filter with normal saline to remove the antimicrobial agent and capture surviving cells on the filter membrane. The filter paper was aseptically placed on a WCA plate, and after incubation, colonies growing on the filter paper were enumerated. Colony counts were expressed as mean log$_{10}$ CFU/ml. For both methods, plates were incubated at 37°C for 48 h under anaerobic conditions, at which time colony counts were performed. These methods have a lower limit of reliable detection of 1 log$_{10}$ CFU/ml. The total change in log$_{10}$ CFU/ml was plotted against time to construct curves summarizing the pharmacodynamic activity of each pharmacokinetic exposure against each organism. Bactericidal activity (99.9% kill) was defined as a $\geq$3-log$_{10}$ CFU/ml decrease in colony count from the initial inoculum. Bacteriostatic activity was defined as a $<$3-log$_{10}$ CFU/ml reduction in colony count from the initial inoculum, while inactivity was defined as growth from the initial inoculum.

**TABLE 1 Susceptibility profiles of strains used in the study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (mg/liter) of a, b</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacteroides fragilisa</em></td>
<td>4.0, 0.125/4, 0.25</td>
</tr>
<tr>
<td><em>Bacteroides fragilisa</em></td>
<td>&gt;32, 2/4, 2</td>
</tr>
<tr>
<td><em>Prevotella bivia</em></td>
<td>16, 0.5/4, 0.25</td>
</tr>
<tr>
<td><em>F. magna</em></td>
<td>0.5, 0.5/4, 1</td>
</tr>
</tbody>
</table>

a ATCC control strain 25285.
b CPT, cefaroline; CPA, cefaroline-avibactam; ERT, ertapenem.
CFU/ml are graphed as a function of time in Fig. 1A to D. The average starting inoculum for each run was 8.1 ± 0.27 log₁₀ CFU/ml. All of the organisms were able to survive and grow under the conditions of the model, as evidenced by the stable growth curves observed for each strain (Fig. 1A to D). Both ertapenem and CPA were bactericidal against all of the strains tested by 72 h, and no emergence of resistance was detected from any of the models. CPA was more active than ertapenem against both B. fragilis strains at the dose-exposures simulated in this study, although this difference did not reach statistical significance for B. fragilis ATCC 25285. The ertapenem simulations had activity similar to that of CPA against F. magna and modestly more activity against P. bivia by 72 h.

**DISCUSSION**

In this study, we evaluated the antibacterial activities of ertapenem and CPA under conditions simulating average human pharmacokinetics in a novel anaerobic PK/PD model. As anticipated, CPA demonstrated potent bactericidal activity *in vitro* at clinically relevant drug exposures. These CPA exposures provided a free time above the MIC of 99 to 100% of the dosing interval for all of the test organisms, correlating with bactericidal activity. Based on what we know about beta-lactams, this high PK/PD ratio is likely above what is necessary to achieve bactericidal activity against these organisms, so it is reasonable to expect a high degree of activity.

**TABLE 2** Pharmacodynamic data of *in vitro* PK/PD model at 72 h

<table>
<thead>
<tr>
<th>Drug or control condition</th>
<th>B. fragilis ATCC 25285</th>
<th>B. fragilis clinical strain</th>
<th>P. bivia clinical isolate</th>
<th>F. magna clinical isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>No-drug growth control</td>
<td>8.28 ± 0.54</td>
<td>9.54 ± 0.31</td>
<td>9.33 ± 0.32</td>
<td>9.21 ± 0.45</td>
</tr>
<tr>
<td>CPA</td>
<td>1.15 ± 0.21</td>
<td>1.95 ± 0.07</td>
<td>2.69 ± 0.05</td>
<td>1.15 ± 0.21</td>
</tr>
<tr>
<td>ERT</td>
<td>3.47 ± 0.81</td>
<td>5.095 ± 1.18</td>
<td>1.0 ± 0.0</td>
<td>1.3 ± 0.42</td>
</tr>
</tbody>
</table>

*P* value<sup>a</sup> 0.055 0.04 0.006 0.92

<sup>a</sup> Two-sided *P* values were used to compare data for CPA and ERT.

**FIG 1** Growth of common anaerobic pathogens *in vitro* PK/PD model system simulating average human pharmacokinetic exposure to ertapenem or ceftaroline-avibactam (CPA) along with a no-drug growth control, shown as changes in log₁₀ CFU/ml graphed as a function of time. (A) *Bacteroides fragilis* ATCC 25285. (B) *Bacteroides fragilis* clinical isolate. (C) *Prevotella bivia* clinical isolate. (D) *Finegoldia magna* clinical isolate.
activity against these pathogens even in the setting of polymicrobial infections, where tissue drug exposures may be less than in the blood (17). While the in vitro PK/PD models demonstrate good activity against these anaerobic pathogens, further clinical evaluation of CPA is warranted. One future direction for clinical research may be in the setting of diabetic foot infections.

Currently, over 25 million Americans have diabetes, and the incidence is increasing. If current trends continue, the CDC estimates that 1 in every 3 Americans will have diabetes by 2050. Between 10 and 20% of these patients will develop a DFI in their mates that 1 in every 3 Americans will have diabetes by 2050. The incidence is increasing. If current trends continue, the CDC estimates that 1 in every 3 Americans will have diabetes by 2050. Between 10 and 20% of these patients will develop a DFI in their lifetime, and more than 50% of these will require some degree of amputation (18). Early recognition and provision of adequate antimicrobial therapy is an important and potentially limb-saving intervention in these infections. The high prevalence of MRSA in the community, the increasing frequency of glycopeptide-nonsusceptible S. aureus strains, such as vancomycin-intermediate S. aureus (VISA) or heterogeneous VISA (hVISA), and the increasing numbers of ESBL-related infections lower the probability of providing adequate empirical coverage with agents such as vancomycin and piperacillin-tazobactam. Cefaroline-fosamil plus avibactam would be the first single-product option for the coverage of MRSA, including hVISA and VISA, as well as anaerobes and enterobacteriaceae, including those that produce broad-spectrum beta-lactamasmes like ESBLs and KPCs (19). This spectrum of activity may be advantageous in the setting of a variety of complicated health care-associated infections, including diabetic foot infections, intra-abdominal infections, and complicated bacterial pneumonias. As important as adequate and responsible provision of antimicrobial therapy is, nonpharmacological interventions like surgical debride-ment and wound care will continue to play important roles in the successful treatment of these complicated infections (8, 20).

This model was designed by our laboratory and has some inherent limitations to its design that should be acknowledged. The anaerobic chambers that the models were fitted into maintained the anaerobic environment immediately around the model, but after each sample was taken, it was transiently exposed to normal atmospheric oxygen levels during the dilution and plating process, which was performed immediately in order to limit this exposure. It is possible, however, that some of the organisms were damaged by this exposure; however, because we were able to consistently observe cell proliferation in our growth control models using these methods, we do not believe that these exposures were substantial enough to alter our results. It is possible, however, that this model would not be suitable to study less aerotolerant organisms.

ACKNOWLEDGMENTS
This work was funded by a grant from Forest Laboratories. M.J.R. has received finding from Cubist, Durata, Forest, Novartis, and Sunovion and funding in part from NIH grant NIAID R21A1092055-01. B.J.W. has nothing to declare.

We thank Forest Laboratories for performing the analytical assays to quantify cefaroline and avibactam levels from the models and for performing susceptibility testing on test organisms.

REFERENCES


