Introduction

Ceftaroline (CPT) demonstrates antibacterial activity against wild-type gram-negative bacteria, but the cephalosporin is inactive against isolates exhibiting extended-spectrum β-lactamases (ESBLs) and other resistance enzymes frequently encountered among gram-negative pathogens. Avibactam (AVI) is a new non-β-lactam β-lactamase inhibitor currently in clinical development in combination with the prodrug, CPT fosamil (Figure 1 and 2). AVI has already shown no antibacterial activity but protects β-lactam agents from hydrolysis in a variety of class A- and class C-producing strains, including:

- ESBL-producing strains
- KPC producers
- AmpC-overexpressing strains

Experimental endocarditis is a reliable model for testing the in vivo efficacy of antibiotics in severe infections. The purpose of the study was to evaluate the in vitro and in vivo activity of CPT alone and in combination with avibactam (CPA) following computer-controlled simulation of injectable human doses of ceftaroline fosamil/avibactam (CXL), compared with that of doripenem (DOR) against an AmpC-producing Enterobacter cloacae using the rabbit model of infective endocarditis with projected human therapeutic doses.

Methods

Susceptibility Testing

MIC Determination. The MICs of CPT, CPA, and DOR were determined in cation-supplemented Mueller-Hinton (MH) broth by the Clinical and Laboratory Standards Institute (CLSI) microdilution technique (1,2). AVI was used at a fixed concentration of 4 mg/L in combination with CPT.

Time-Kill Curves. Killing experiments were performed to evaluate the in vitro antibacterial activity of CPT, CPA, and DOR against E. cloacae. Time-kill curves were performed in glass flasks containing MH broth, using an inoculum of 5 x 10⁶ colony-forming units (CFU) per mL. AVI was used at a fixed concentration of 4 mg/L in combination with CPT. Surviving bacteria were counted after 0, 3, 6, and 24 hours of incubation at 37°C by subculturing 50 μL serial dilutions of samples (in 0.9% sodium chloride) on agar plates using a spiral plater (Spiral System; Interscience, Saint-Nom-La-Bretèche, France) to avoid potential carry-over effect. A bactericidal effect was defined as a 3-log₉ CFU/mL decrease compared with the initial inoculum after incubation.

Pharmacokinetic Studies

A CPT pharmacokinetic simulation was previously performed and validated (2). Blood samples were taken from 3 healthy rabbits at 0, 0.25, 0.5, 1, 1.5, 2, 3, and 4 hours after the end of the administration of a 1-hour infusion of AVI (30 mg/kg). The pharmacokinetic data were processed and compared with those for human subjects to determine an equivalent dose. AVI was used at a fixed concentration of 4 mg/L in combination with CPT. Surviving bacteria were counted after 0, 3, 6, and 24 hours of incubation at 37°C by subculturing 50 μL serial dilutions of samples (in 0.9% sodium chloride) on agar plates using a spiral plater (Spiral System; Interscience, Saint-Nom-La-Bretèche, France) to avoid potential carry-over effect. A bactericidal effect was defined as a 3-log₉ CFU/mL decrease compared with the initial inoculum after 24 hours of incubation.

Animal Model (endocarditis)

A catheter was placed into the left ventricle of anesthetized New Zealand White rabbits. After 24 hours, endocarditis was induced with an inoculum of 1 x 10⁹ CFU/mL E. cloacae. Treatment began 24 hours after inoculation using a computer-controlled pump, as described more fully elsewhere (1). Aortic valve vegetations were excised, weighed, and then homogenized in 0.5 mL of saline. Vegetation homogenates were spread on agar plates containing the study drugs at concentrations corresponding to 4-fold the MIC. Bacterial counts were determined after 48 hours of incubation at 37°C.

Pharmacokinetic Studies

A CPT pharmacokinetic simulation was previously performed and validated (2). Blood samples were taken from 3 healthy rabbits at 0, 0.25, 0.5, 1, 1.5, 2, 3, and 4 hours after the end of the administration of a 1-hour infusion of AVI (30 mg/kg). The pharmacokinetic data were processed and compared with those for human subjects to determine an equivalent dose. AVI was used at a fixed concentration of 4 mg/L in combination with CPT. Surviving bacteria were counted after 0, 3, 6, and 24 hours of incubation at 37°C by subculturing 50 μL serial dilutions of samples (in 0.9% sodium chloride) on agar plates using a spiral plater (Spiral System; Interscience, Saint-Nom-La-Bretèche, France) to avoid potential carry-over effect. A bactericidal effect was defined as a 3-log₉ CFU/mL decrease compared with the initial inoculum after 24 hours of incubation.

Therapeutic Regimens

For each strain, animals were randomly assigned to either no treatment (controls), CPT fosamil regimen mimicking the human dose of 10 mg/kg/6h (300 mg q8h), CXL regimen mimicking the human dose of 10 mg/kg/6h (300 mg q8h), and DOR regimen mimicking the human dose of 8.3 mg/kg/6h (500 mg q8h).

Results

MICs for the AmpC-overexpressing E. cloacae isolate are shown in Table 1. The results of the time-kill curve studies are shown in Figure 3 (CPT and CPA) and Figure 4 (DOR). CPT alone was not active against an AmpC-producing E. cloacae strain (CPT MIC = 128 mg/L). In combination with a fixed concentration of AVI (4 μg/mL), CPT demonstrated bactericidal activity after 24 hours of incubation (from 4 to 32 mg/L). After administration of AVI into the rabbit, the maximum plasma concentration (Cₘₚ) was ca. 29 mg/L, with a half-life <20 min. Because of the very short, spontaneous half-life of the inhibitor in rabbits, simulation was required to reach valid conclusions relative to human applications. The concentrations of each AVI and CPT obtained after computer-controlled simulation are shown in Figure 5. In vivo results after a 4-day treatment regimen are shown in Figure 6. No mutants resistant to CPT, CPA, or DOR were detected after a 4-day treatment using the endocarditis model.

Table 1. MICs of CPA, CPT, and DOR for the AmpC-Producing E. cloacae Isolate

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (mg/L)</th>
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<tbody>
<tr>
<td>CPA</td>
<td>1</td>
</tr>
<tr>
<td>CPT</td>
<td>0.064</td>
</tr>
<tr>
<td>DOR</td>
<td>1</td>
</tr>
</tbody>
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Discussion/Conclusion

- As expected with an MIC of 128 mg/L, CPT failed to exhibit an effect against an AmpC-overexpressing isolate after a 4-day treatment (>P>0.5 vs control animals). CPA showed a bactericidal effect (>3-log CFU/cm³ decrease of vegetations) after 4 days of treatment using a simulated human-equivalent dosage in this experimental model
- Although DOR displayed a lower MIC against E. cloacae, the carbapenem did not demonstrate better efficacy in vivo
- In this experimental model of E. cloacae endocarditis, the combination of CPT with the new non-β-lactam β-lactamase inhibitor, AVI, had equivalent efficacy to that of the carbapenem, doripenem, in terms of reducing bacterial load in vegetations
- These data demonstrate the ability of AVI to (i) protect CPT in vitro and in vivo, and (ii) improve the in vivo activity of CPT against otherwise nonsusceptible pathogens. Results strongly support the addition of AVI to CPT as an effective strategy against AmpC-overexpressing isolates
- CXL could represent a promising alternate therapeutic option to treat multidrug-resistant gram-negative severe infections and may provide a therapeutic alternative to carbapenems.

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References