Efficacy of Ceftaroline Fosamil in Combination With the Non-β-Lactam β-Lactamase Inhibitor Avibactam Against AmpC-Producing *Enterobacter cloacae*: Comparative Study With Doripenem in a Rabbit Experimental Endocarditis Model

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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 (Figures 1 and 2). AVI has virtually no intrinsic 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 simulated 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.

Figure 1. Ceftaroline Fosamil

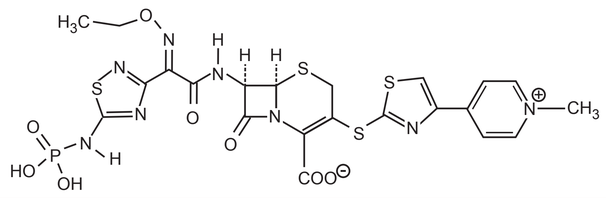
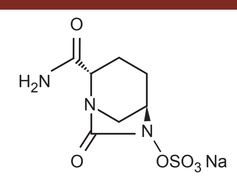


Figure 2. Avibactam



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 x10^6 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-$\log_{10}$ 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 10^9 CFU *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 buffer and used for quantitative cultures on agar for 24 hours at 37°C. To evaluate whether CPT, CPA, or DOR treatment could induce the selection of resistant variants, undiluted 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, 0.75, 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 humans. A computer-controlled system was then used to obtain the human kinetic profiles for AVI in rabbits. Simulation was intended to provide pharmacokinetic parameters close to those observed in healthy volunteers after administration of a single 600-mg bolus (ca. 10 mg/kg). A total dose of 60 mg/kg needed to be infused into the rabbit over an 8-hour period in order to simulate the kinetics in human serum after the administration of a 10 mg/kg dose.

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/8h (600 mg q8h), CXL regimen mimicking the human dose of 10 mg/kg/8h (600 mg q8h), and DOR regimen mimicking the human dose of 8.3 mg/kg/8h (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 (C_{max}) 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 CPT, CPA, and DOR for the AmpC-Producing *E. cloacae* Isolate

	MIC (mg/L)		
	CPT	CPA	DOR
AmpC-*E. cloacae*	128	1	0.064

CPT = ceftaroline; CPA = ceftaroline combined with avibactam; DOR = doripenem.

Figure 3. In Vitro Activity of CPT at 32 mg/L (CPT 32) and CPA at 1 mg/L (CPT 1), 4 mg/L (CPT 4), 8 mg/L (CPT 8), and 32 mg/L (CPT 32) Against AmpC-Producing *E. cloacae* (fixed AVI concentration of 4 mg/L)

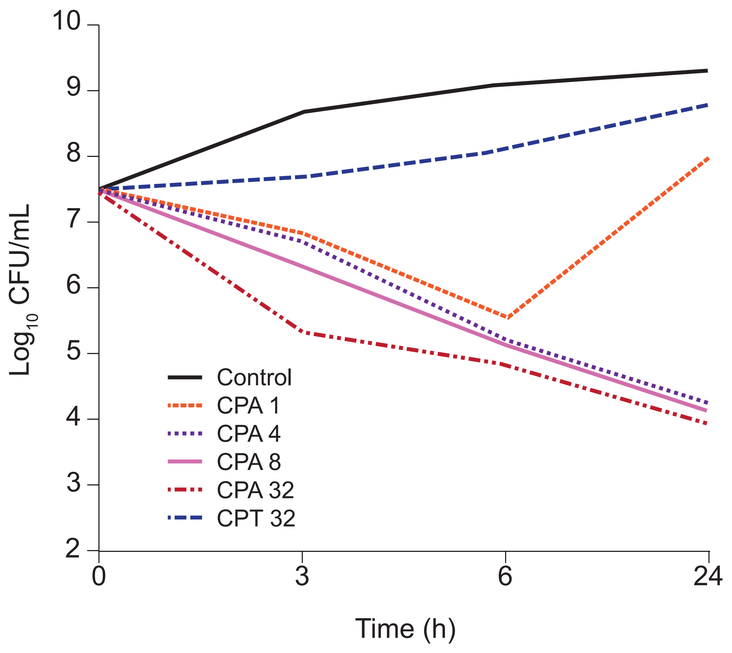


Figure 4. In Vitro Activity of DOR at 1 mg/L (DOR 1), 4 mg/L (DOR 4), 8 mg/L (DOR 8), and 32 mg/L (DOR 32) Against AmpC-Producing *E. cloacae*

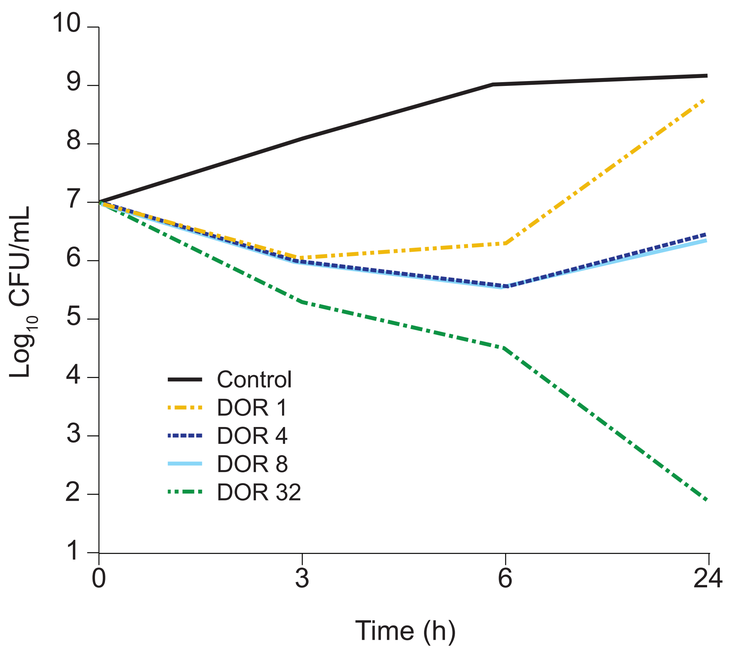


Figure 5. Experimental Pharmacokinetics of CPT (mimicking the human dose of 10 mg/kg/8h [600 mg q8h]) and AVI (mimicking the human dose of 10 mg/kg/8h [600 mg q8h]) in Animal Plasma

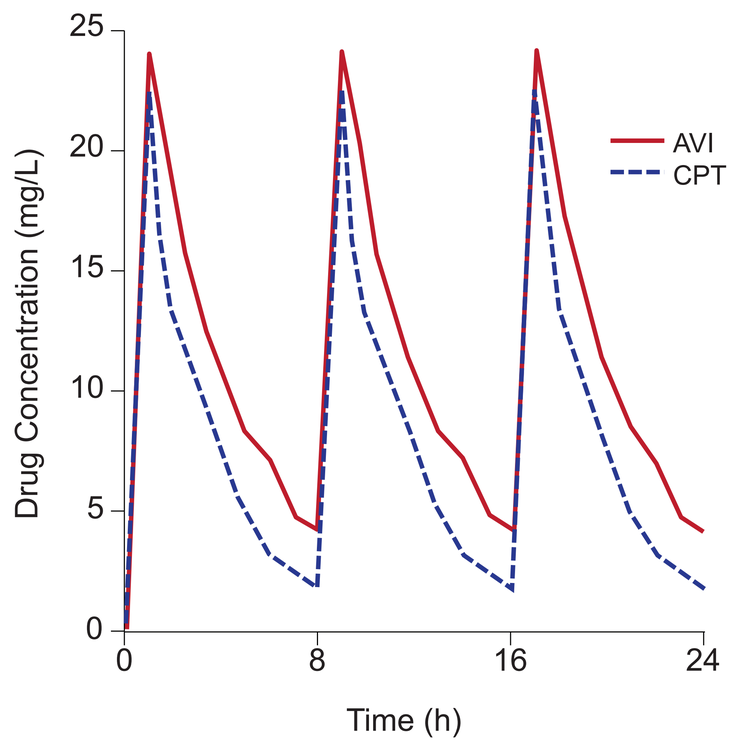
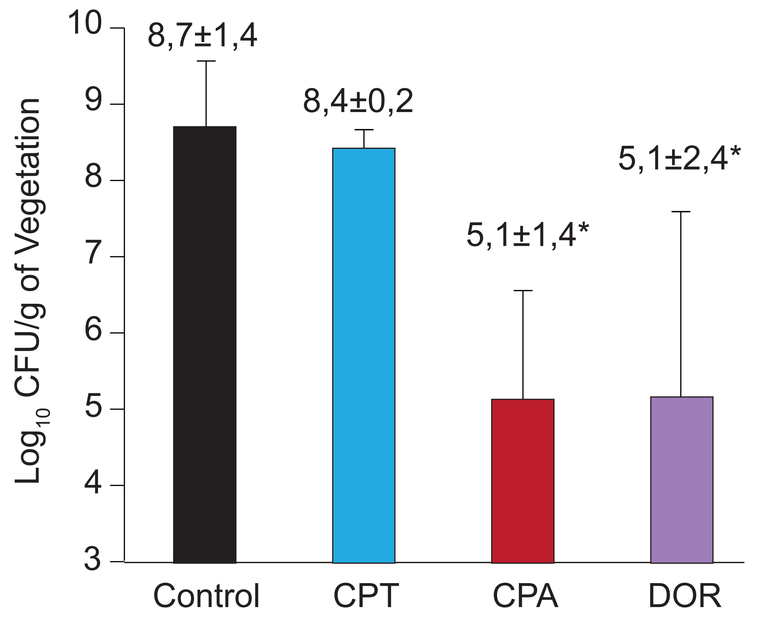


Figure 6. Bacterial Titers in Vegetations After 4 Days of Treatment



Error bars represent standard deviations.
*P<0.001 vs control animals and CPT fosamil-treated animals.

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>0.05 vs control animals). CPA showed a bactericidal effect (>3-$\log_{10}$ CFU/g 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.

References

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