Pharmacokinetics-Pharmacodynamics of CB-618 in Combination with Cefepime, Ceftazidime, Ceftolozane, or Meropenem: the Pharmacological Basis for a Stand-Alone β -Lactamase Inhibitor

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ABSTRACT A major challenge in treating patients is the selection of the “right” antibiotic regimen. Given that the optimal β -lactam/ β -lactamase inhibitor pair is dependent upon the spectrum of β -lactamase enzymes produced and the frequency of resistance to the β -lactamase inhibitor, it might be useful if a stand-alone were available for the clinician to pair with the “right” β -lactam rather than only in a fixed combination. We describe herein a one-compartment *in vitro* infection model studies conducted to identify the magnitudes of the pharmacokinetic-pharmacodynamic (PK-PD) index for a β -lactamase inhibitor, CB-618, that would restore the activity of four β -lactam partner agents (cefepime, ceftazidime, ceftolozane, and meropenem) with various doses (1 or 2 g) and dosing intervals (8 or 12 h). The challenge panel included *Klebsiella pneumoniae* ($n = 5$), *Escherichia coli* ($n = 2$), and *Enterobacter cloacae* ($n = 1$) strains, which produced a wide variety of β -lactamase enzymes (AmpC, CTXM-15, KPC-2, KPC-3, FOX-5, OXA-1/30, OXA-48, SHV-1, SHV-11, SHV-27, and TEM-1). Free-drug human concentration-time profiles were simulated for each agent, and specimens were collected for drug concentration and bacterial density determinations. CB-618 restored the activity of each β -lactam partner. The magnitudes of the CB-618 ratio of the area under the concentration-time curve from 0 to 24 h to the MIC (i.e., the AUC/MIC ratio) associated with net bacterial stasis and 1- and 2-log₁₀ CFU/ml reductions from baseline at 24 h were 11.2, 32.9, and 136.3, respectively. These data may provide a PK-PD basis for the development of a stand-alone β -lactamase inhibitor.

KEYWORDS β -lactamase inhibitor, CB-618, pharmacokinetics-pharmacodynamics

The clinical challenge posed by β -lactamase-producing *Enterobacteriaceae* is complex and multidimensional. The complexity is due to various degrees of drug efflux pump and porin expression and β -lactamase enzyme production (1–3). Moreover, these bacteria typically produce multiple β -lactamase enzymes, to which the β -lactam substrate shows differing degrees of stability (3).

For instance, the vast majority New Delhi metallo- β -lactamase-type (NDM)-producing *Escherichia coli* elaborate other β -lactamase enzymes (e.g., extended-spectrum β -lactamase [ESBL], TEM, and SHV types) (4). Although aztreonam is resistant to hydrolysis by NDM-type β -lactamases, it is susceptible to many other common β -lactamase enzymes. Consequently, to effectively treat NDM-type-producing *E. coli* with aztreonam requires the addition of a β -lactamase inhibitor to prevent its hydrolysis by β -lactamases other than NDM.

Another dimension to the challenge involves the consequence of the on-therapy bacterial resistance development to the β -lactamase inhibitor. For example, the intrinsic

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TABLE 1 Known β -lactamase enzymes and susceptibilities of each challenge isolate to CB-618 and each β -lactam, alone and in combination, using a fixed concentration of 4 mg/liter

		Microbroth MIC (mg/liter) ^a								
Clinical isolate	Known β -lactamase enzyme(s)	CB-618	MER alone	MER + CB-618	FEP alone	FEP + CB-618	TOL alone	TOL + CB-618	CAZ alone	CAZ + CB-618
<i>K. pneumoniae</i>										
79	KPC-3, SHV-11, TEM-1, FOX-5	256	128	1	256	2	256	4	–	–
908	KPC-2, SHV-27, TEM-1	256	64	0.5	32	0.5	128	1	–	–
6501	KPC-3, SHV-11, TEM-1	256	512	2	256	2	–	–	>512	2
9380	KPC-2, TEM-1, SHV-1	256	–	–	–	–	32	1	–	–
4192	OXA-48	>512	16	0.25	–	–	–	–	–	–
<i>E. coli</i>										
4189	OXA-48	>512	64	0.5	–	–	–	–	–	–
4643	CTX-M-15, OXA-1/30	>512	–	–	>256	0.03	–	–	64	0.25
<i>E. cloacae</i> 4182										
	AmpC derepressed	256	–	–	–	–	–	–	256	1

^aMER, meropenem; FEP, cefepime; TOL, ceftolozane; CAZ, ceftazidime. All combination MIC values were determined using a fixed 4-mg/liter concentration of CB-618.

–, Not applicable since these combinations were not evaluated.

sic stability of meropenem and cefepime to OXA-48-type carbapenemase is different (1, 5). If on-therapy bacterial resistance develops to a β -lactamase inhibitor active against OXA-48-type paired with meropenem or cefepime, the MIC of the meropenem combination usually increases to values of 64 to 128 mg/liter, while that for the cefepime combination increases to 16 to 32 mg/liter. Given that the typical maximal serum concentrations of meropenem and cefepime following 2-g intravenous doses are 60 and 150 mg/liter (6, 7), respectively, the resultant percentage of time that concentrations were above the MIC (%T>MIC) for meropenem-containing regimens approaches zero, but potentially with some drug effect for cefepime-containing regimens. In such a circumstance, the consequence to the patient treated with a meropenem-containing regimen could be catastrophic.

Part of the challenge in effectively treating individual patients is the selection of the “right” antibiotic regimen. Given that the optimal β -lactam/ β -lactamase inhibitor pair is dependent upon the spectrum of β -lactamase enzymes produced and the resistance to the β -lactamase inhibitor, it might be useful if a standalone β -lactamase inhibitor were available for the clinician to pair with the “right” β -lactam rather than only in a fixed combination. To this end, the studies using a one-compartment *in vitro* infection model described herein were designed to identify the magnitudes of the PK-PD index for CB-618, a non- β -lactam diazabicyclooctane class β -lactamase inhibitor, that would be sufficient to rescue the activity of multiple β -lactam partner agents with various doses and dosing schedules.

RESULTS

***In vitro* susceptibility testing.** The susceptibility study results for CB-618, cefepime, ceftazidime, ceftolozane, and meropenem and each β -lactam in combination with CB-618 against each challenge isolate are presented in Table 1. Collectively, these clinical isolates produced a wide range of β -lactamase enzymes, including carbapenemases, cephamycinases, and broad- and extended-spectrum β -lactamases (KPC-2, KPC-3, FOX-5, OXA-48, OXA1/30, SHV-11, SHV-27, CTX-M-15, AmpC, and TEM-1). As expected, CB-618 alone displayed little microbiological activity, as evidenced by MIC values of ≥ 256 mg/liter across isolates. Each challenge isolate was categorized as resistant to the β -lactam agent alone against which it was tested. However, when each β -lactam was tested in combination with CB-618, the MIC values decreased markedly, with MIC values generally at the upper margin of the susceptible category for the β -lactam agent alone (8).

Pharmacokinetics. Figure 1 shows the relationships between observed and targeted CB-618, meropenem, cefepime, ceftolozane, and ceftazidime concentrations. As evidenced by the agreement between observed and targeted concentrations for each

FIG 1 Relationships between observed and targeted CB-618, meropenem, cefepime, ceftolozane, and ceftazidime concentrations.

drug (CB-618: $r^2 = 0.996$, slope = 0.934, and intercept = -0.457 ; meropenem: $r^2 = 0.990$, slope = 0.869, and intercept = -0.086 ; cefepime: $r^2 = 0.987$, slope = 0.929, and intercept = -1.61 ; ceftolozane: $r^2 = 0.980$, slope = 0.889, and intercept = -1.07 ; ceftazidime: $r^2 = 0.946$, slope = 0.870, and intercept = 2.38), the pharmacokinetic profiles were simulated well in the one-compartment *in vitro* infection model for all dosing regimens evaluated.

Dose-ranging studies. The dose-ranging studies provided insight about the inter-isolate variability associated with CB-618 efficacy when administered in combination with multiple β -lactam agents. In all studies, the results for the no-treatment control regimens were as expected, producing uninhibited growth over a 24-h period. The active control arms representing monotherapy β -lactam regimens achieved initial bactericidal activity against most isolates, followed by immediate regrowth matching that of the no-treatment growth control at 24 h. The β -lactam/ β -lactamase inhibitor combination regimens provided a full range of effect from those increasing in bacterial burden and matching that of the no-treatment control arms to $>6 \log_{10}$ CFU/ml reductions in bacterial burden over a 24-h period.

PK-PD analyses. The relationships between change from baseline in $\log_{10}$ CFU/ml at 24 h and the CB-618 area under the concentration-time curve (AUC) from 0 to 24 h to the MIC (AUC/MIC ratio) based on the potentiated MIC values for meropenem alone, the pooled cephalosporins, and all β -lactams pooled, as well as the data for CB-618 in combination with these agents, are shown in Fig. 2, 3, and 4, respectively. When we examined the combination of CB-618 with meropenem, the magnitudes of the CB-618 AUC/MIC ratio associated with net bacterial stasis and the 1- and 2- $\log_{10}$ CFU/ml reductions from the baseline at 24 h (Fig. 2) were 27.8, 86.2, and 416, respectively. The parameter estimates (standard error) for the Hill model describing the relationship between change from baseline in $\log_{10}$ CFU/ml at 24 h and CB-618 AUC/MIC ratio

FIG 2 Relationship between change in $\log_{10}$ CFU/ml from baseline at 24 h and CB-618 AUC/MIC ratio, utilizing the CB-618 potentiated β -lactam MIC, based on comodeling of data for all clinical isolates and CB-618 in combination with meropenem.

shown in Fig. 2 were 3.28 (1.04), 6.17 (1.92), 0.609 (0.280), and 22.7 (10.8) for the change in $\log_{10}$ CFU/ml at 24 h in the absence of drug (E_0), the maximum change in $\log_{10}$ CFU/ml from E_0 (E_{max}), the Hill coefficient, and the CB-618 AUC/MIC ratio associated with a half-maximal effect (EC_{50}), respectively.

When all cephalosporins were pooled (Fig. 3), the magnitudes of the CB-618 AUC/MIC ratio associated with net bacterial stasis and the 1- and 2- $\log_{10}$ CFU/ml reductions from baseline at 24 h were lower than those observed for meropenem, achieving values of 7.23, 17.9, and 64.5, respectively. The parameter estimates (standard error) for the Hill model describing the relationship between change from the baseline in $\log_{10}$ CFU/ml at 24 h and CB-618 AUC/MIC ratio shown in Fig. 3 were 6.00 (3.68), 9.17 (4.00), 0.586 (0.216), and 2.44 (3.37) for E_0 , E_{max} , the Hill coefficient, and the EC_{50} , respectively.

FIG 3 Relationship between change in $\log_{10}$ CFU/ml from baseline at 24 h and CB-618 AUC/MIC ratio, utilizing the CB-618 potentiated β -lactam MIC, based on comodeling of data for all clinical isolates and CB-618 in combination with the cephalosporin partners.

FIG 4 Relationship between change in $\log_{10}$ CFU/ml from baseline at 24 h and CB-618 AUC/MIC ratio, utilizing the CB-618 potentiated β -lactam MIC, based on comodeling of data for all clinical isolates and CB-618 in combination with β -lactam partners.

Figure 4 describes the efficacy of CB-618 across four different β -lactam partners, eight different clinical isolates producing 11 different β -lactamase enzymes, two different doses, and two different dosing intervals. The magnitudes of the CB-618 AUC/MIC ratio associated with net bacterial stasis and 1- and 2- $\log_{10}$ CFU/ml reductions from baseline at 24 h across all beta lactams (Fig. 4) were 11.2, 32.9, and 136.3, respectively. The parameter estimates (standard error) for the Hill model describing the relationship between change from baseline in $\log_{10}$ CFU/ml at 24 h and CB-618 AUC/MIC ratio shown in Fig. 4 were 6.00 (3.57), 9.47 (4.18), 0.458 (0.191), and 3.39 (5.09) for E_0 , E_{max} , the Hill coefficient, and the EC_{50} , respectively.

DISCUSSION

The goal of the one-compartment *in vitro* infection model studies described herein was to identify a CB-618 PK-PD index sufficient to rescue the activity of multiple β -lactam partner agents with various doses and dosing schedules. The CB-618 PK-PD index (CB-618 AUC₄/MIC ratio, applying the CB-618 potentiated β -lactam MIC) utilized in this investigation was selected based upon a previous dose fractionation study (9). In that study, fractionated CB-618 doses were evaluated in the context of meropenem administered 1 g every 8 h as a 1-h infusion against a KPC-3-producing *K. pneumoniae* clinical isolate. CB-618 AUC/MIC ratio ($r^2 = 0.835$) and CB-618 C_{max} /MIC ratio ($r^2 = 0.826$) better described efficacy than CB-618 %T_{>threshold} ($r^2 = 0.792$). The CB-618 AUC/MIC ratio was chosen over CB-618 C_{max} /MIC ratio for dose optimization because AUC can be better estimated in patients due to the transient nature of C_{max} .

Across eight different CB-618-containing regimens, which included four different β -lactams and two different dose levels and dosing intervals against eight *Enterobacteriaceae* isolates producing a wide variety of β -lactamases (Table 1), CB-618 restored the bactericidal activity of each β -lactam partner (Fig. 4). The magnitudes of the CB-618 AUC/MIC ratio associated with net bacterial stasis and the 1- and 2- $\log_{10}$ CFU/ml reductions from baseline at 24 h were 11.2, 32.9, and 136.3, respectively.

Interestingly, over a wide range of CB-618 AUC/MIC ratios, cephalosporin-containing regimens outperformed meropenem-containing regimens in terms of bacterial kill. This may indicate that there is room for meropenem dose regimen optimization. That is, higher meropenem doses or prolonged infusion durations likely would have resulted in a greater bacterial kill. Notwithstanding, these data may provide a PK-PD basis for the development of a stand-alone β -lactamase inhibitor.

A stand-alone β -lactamase inhibitor may be useful to combat the rising tide of β -lactamase-producing bacteria. The current strategy for using β -lactamase inhibitors is to pair the β -lactamase inhibitor in a fixed combination with a single β -lactam. This strategy may limit the potential utility of novel broad-spectrum β -lactamase inhibitors. This is especially apparent when one recognizes that the optimal β -lactam/ β -lactamase inhibitor pair is dependent upon the spectrum of β -lactamase enzymes produced and the frequency of resistance to the β -lactam/ β -lactamase inhibitor combination. A stand-alone β -lactamase inhibitor would allow the clinician to pair the inhibitor with the optimal β -lactam partner for a given clinical situation.

Clearly, a stand-alone β -lactamase inhibitor may pose some clinical challenges. For instance, there may be an increased risk for medical errors as a result of inappropriate β -lactam/ β -lactamase inhibitor pairing, while another challenge may be failure to administer either the β -lactam or β -lactamase inhibitor. Either of these medical errors would be catastrophic for the patient.

There are a number of strengths of the studies described herein. Perhaps foremost among these was the inclusion of multiple β -lactam partner agents, each with varying intrinsic stability to β -lactamase enzymes produced by the challenge isolates. Moreover, the challenge isolates included three clinically relevant species (*Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae*) that together produced 11 different β -lactamase enzymes (Table 1).

There are two main limitations of these studies. First, the one-compartment *in vitro* infection models were carried out for 24 h. This study duration was insufficient to evaluate the effect of regimen exposure intensity and therapy duration on the amplification of preexisting drug-resistant bacterial subpopulations. Second, the *in vitro* infection models utilized do not account for the components of the immune system and were conducted using Mueller-Hinton broth medium that optimizes bacterial growth. Additional studies utilizing a hollow-fiber *in vitro* infection model and immunocompetent and immunosuppressed animal infection models are needed to address these limitations.

In conclusion, we successfully identified the magnitudes of the CB-618 AUC/MIC ratio in combination with eight different β -lactam regimens that restored the activity of each β -lactam partner. CB-618 AUC/MIC ratios of 136.3 and greater were associated with a 2-log₁₀ CFU/ml reduction from baseline. These data may provide a PK-PD basis for the development of a stand-alone β -lactamase inhibitor.

MATERIALS AND METHODS

Bacteria, β -lactam agents, and β -lactamase inhibitor. A total of eight serine carbapenemase-producing clinical isolates were used in the studies described here. These isolates were obtained from JMI Laboratories (North Liberty, IA) and included *E. cloacae* (1), *E. coli* (2), and *K. pneumoniae* (5). Isolates were selected for use in these studies based upon their elevated MIC values to cefepime, ceftazidime, ceftolozane, and meropenem alone and in combination with CB-618. β -Lactamase production was confirmed by JMI Laboratories using PCR, sequencing, and isoelectric focusing according to standard methodologies. CB-618, cefepime, ceftazidime, ceftolozane, and meropenem were provided by Merck and Co. (Kenilworth, NJ).

Media and *in vitro* susceptibility studies. Susceptibility studies were completed in triplicate over a 2-day period in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (10). Susceptibility to CB-618, cefepime, ceftazidime, ceftolozane, and meropenem were determined by broth and agar dilution methodologies using cation-adjusted Mueller-Hinton growth medium (BD Laboratories, Franklin Lakes, NJ). The susceptibility of each challenge isolate was determined for each β -lactam alone and in combination with a fixed CB-618 concentration (4 mg/liter). The modal MIC values were utilized in all analyses.

One-compartment *in vitro* infection model and sample processing. A previously described one-compartment *in vitro* infection model was utilized in all dose-ranging studies (11, 12). The one-compartment *in vitro* infection model consisted of a central infection compartment set upon a magnetic stir plate within a humidified incubated at 35°C. Inside the central compartment, a suspension of the challenge isolate was constantly stirred to ensure a homogeneous mixture of bacteria and was exposed to β -lactam and β -lactamase inhibitor concentrations simulating human free-drug pharmacokinetic exposures. Through the use of computer-controlled syringe pumps, free-drug concentration-time profiles were simulated for each study drug. Specimens for determination of bacterial density and drug concentration were collected from the central infection compartment at predetermined time points (provided below).

In these experiments, bacterial suspensions of $\sim 10^6$ CFU/ml were prepared for each challenge isolate using cultures grown overnight on Trypticase soy agar plates supplemented with 5% sheep blood (BD

Laboratories). Prior to addition to the central compartment, random colonies were collected from the plates and grown to a mid-logarithmic phase in Mueller-Hinton broth at 35°C and 125 rpm in a shaking water bath. The bacterial concentration within each flask was determined from the optical density at 630 nm, for each challenge isolate, and then adjusted to achieve a target initial inoculum of 10^6 CFU/ml with fresh Mueller-Hinton medium. The bacteria were exposed to changing free-drug concentrations of CB-618, cefepime, ceftazidime, ceftolozane, and meropenem, which simulated that observed in humans based on the following half-lives (protein binding percentage): meropenem, 1 h (2%) (13); cefepime, 2.2 h (20%) (6); ceftolozane, 1.8 h (16%) (14); ceftazidime, 2 h (10%) (15); and CB-618, 1.8 h (15%) (Merck & Co., Kenilworth, NJ, USA) (unpublished data).

At baseline and 2, 4, 8, 12, and 24 h after therapy initiation, 1-ml samples were collected for bacterial density determination. Each sample was centrifuged, washed, and resuspended with sterile normal saline twice to prevent drug carryover. The washed samples were serially diluted in sterile normal saline and cultured onto drug-free Trypticase soy agar enriched with 5% sheep blood. Plated samples were then placed in a humidified incubator at 35°C for 24 h, and then the bacterial density was determined. In addition, 1-ml samples were collected for drug concentration determination at 1, 3, 5, 7, 9, 11, 13, and 23 h after initiation of therapy for each study. All samples collected for drug concentration determination were immediately frozen at -80°C until assayed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

CB-618 dose-ranging studies. A range of CB-618 doses infused over 1 h, representing AUC values over 0 to 24 h of 2.27 to 581 mg·h/liter were studied in the context of a typical β -lactam regimen for the agents evaluated. The dosing regimens for β -lactam agents were either 1 or 2 g administered either every 8 or 12 h as a 1-h infusion. Each CB-618- β -lactam combination regimen was evaluated against a minimum of three of the challenge isolates and compared to the no-treatment control. All studies were performed in duplicate.

PK-PD analysis. Data from each CB-618 dose-ranging study were pooled and evaluated using a Hill-type model and nonlinear least-squares regression. The independent variable in this analysis was the PK-PD index for CB-618, which was the CB-618 AUC/MIC ratio. The choice of this PK-PD index was based on data from a previous dose fractionation study with CB-618 (9). The dependent variable was change in bacterial density from baseline at 24 h. The magnitudes of the CB-618 AUC/MIC ratio associated with net bacterial stasis, as well as 1- and 2-log₁₀ CFU/ml reductions from the baseline at 24 h, were determined.

Analytical method. Each sample was assayed by LC-MS/MS (AB Sciex, Framingham, MA). The standard curves for CB-618, cefepime, ceftazidime, and meropenem were linear over a concentration range of 0.025 to 50 mg/liter. For ceftolozane, the standard curve was linear for a concentration range of 0.1 to 80 mg/liter. The interday coefficients of variation for CB-618, cefepime, ceftazidime, ceftolozane, and meropenem quality control samples ranged between 0.4 and 9.4%, 2.5 and 6.7%, 1.3 and 11.4%, 1.1 and 3.8%, and 0.2 and 11.6%, for each agent, respectively.

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