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Pharmacokinetic driver of avibactam effect against β-lactamase-producing Enterobacteriaceae established in an *in vitro* pharmacokinetic model of infection

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Revised Abstract

Background: The dominant pharmacokinetic driver of β-lactamase inhibitor activity remains in doubt even though T>threshold has been suggested. We used a large data set collected while supporting the development of ceftaroline (CPT) plus avibactam (AVI) to investigate which pharmacokinetic measure best correlated to antibacterial effect against β-lactam-producing Enterobacteriaceae.

Methods: A dilutional single-compartment *in vitro* model was used. Simulations were performed over 48 h. Three strains were used: *E. coli* CTX-M-producer (CPT+AVI MIC 0.08 mg/L); *E. cloacae* AmpC-hyperproducer (CPT+AVI MIC 1.8 mg/L); *K. pneumoniae* KPC-producer (CPT+AVI MIC 3.6 mg/L). Two data sets employed a series of dose ranging simulations AUC 0 to 240 mg/L AVI (8 exposures), plus 2–3 fractionation experiments. In all experiments CPT was modelled at a) standard 600 mg 8-hourly dosing, or b) an enhanced data set where CPT was dosed at 600 mg 12-hourly or 8-hourly. AVI was dosed at 600 mg 12-hourly, 600 mg 8-hourly, 1200 mg 24-hourly and 1800 mg 24-hourly. Antibacterial effect was assessed by change in viable count from time zero at 24 h (d24 log CFU/mL). PK measure and d24 were related using Sigmoid E_{max} equation.

Results: In the initial data set were 20 experiments with CTX-M strain, 35 with the AmpC strain and 20 experiments with the KPC strain. AUC, C_{max} T≥1 mg/L, T≥2 mg/L, T≥4 mg/L were related to d24. For AUC R^2 ranged from 0.672–0.978, C_{max} 0.746–0.845, T≥1 0.26–0.398; T≥2 0.188–0.542, T≥4 0.168–0.291. For two strains (CTX-M, AmpC-producers) C_{max} correlated best to d24, while for the KPC-producer, AUC was best. A similar pattern was observed with the enhanced data set (CTX-M, 35 experiments; AmpC, 42 experiments; KPC, 36 experiments), R^2 values were lower but C_{max} was best correlated to d24 for the CTX-M and KPC strain, and AUC for the AmpC-hyperproducer. A pooled analysis of all strains indicated an R^2 of 0.506 for AUC and 0.563 for C_{max}.

Conclusions: For AVI antibacterial effect C_{max} and AUC best correlated with d24. The closeness of correlation was dependent on data set and strain. T≥ threshold had a uniformly poor relationship to antibacterial effect.

Background

Avibactam is a non-β-lactam β-lactamase inhibitor, chemically unrelated to the β-lactamase inhibitors currently used in clinical practice. Avibactam inhibits Class A, C and some D β-lactamases including TEM, SHV, ESBLs, AmpC-hyperproducers and *Klebsiella pneumoniae* carbapenemases (KPC), but does not inhibit IMP or VIM metallo-β-lactamases.

The combination of ceftaroline (CPT) and avibactam, at a fixed concentration of avibactam at 2 mg/L, reduces Enterobacteriaceae MICs to ≤2 mg/L in strains with CPT MIC of >128 mg/L, due to the presence of Class A, C, ESBL and D enzymes or combinations of the above. Avibactam has no antimicrobial activity on its own, with MIC_{50} values being for the most part ≥32 mg/L against different species of Enterobacteriaceae (*Escherichia coli*, MIC_{50} 8 mg/L, *K. pneumoniae* MIC_{50} 16 mg/L).

Single- and multiple-dose pharmacokinetic studies have been performed with CPT at a range of doses from 250 mg to 1000 mg. Pharmacokinetics are linear over this dose range with a C_{max} of 9.9 mg/L following a 250 mg single dose, 23 mg/L after a 750 mg dose, and 30.2 mg/L following a 1000 mg/L dose. Multiple doses of 600 mg infused over 1 h every 12 h daily for 14 days produced peak concentration of 19 mg/L (day 1) and 21 mg (day 14) with a half-life of 2.5 h and $AUC_{0\text{-}12}$ of 56 mg/L.h. Protein binding in human plasma is <10% in the concentration range 50–150 mg/L.

The pharmacokinetics of avibactam as a single dose in healthy volunteers has also been reported. C_{max} increased in a dose proportional manner from 2.7 mg/L at 50 mg to 124 mg/L at 2000 mg. The half-life was 1.5–2.7 h.

CPT has typical cephalosporin pharmacodynamics, being T>MIC driven, and a free drug T>MIC of 39.7 ± 15.7% producing a 24 h net bacteriostatic effect for Enterobacteriaceae in our *in vitro* model.

The objective of the present work was to use an existing data set of dosing simulations with CPT plus avibactam to try to establish for three strains of Enterobacteriaceae if the pharmacokinetic driver of effect for avibactam could be established.

Materials and Methods

In vitro pharmacokinetic model

A Fermac 301 Fermentation System (ElectroLab, Tewkesbury, England) *in vitro* pharmacokinetic model was used to simulate free drug serum concentrations of CPT associated with 400 mg 8-hourly, 600 mg intravenous 12-hourly and 600 mg 8-hourly dosing in man. Avibactam was simulated at free drug concentrations associated with doses of 600 mg as well as continuous infusions at 0.5–10 mg/L and several dose fractionations at AUC_{24} between 1.2 to 72 mg/L.h.

Media

100% cation supplemented Mueller-Hinton Broth (MHB) was used in all experiments. Nutrient agar plates (Oxoid, Basingstoke, England) were used to recover bacterial strains from the model. Five microlitres β-lactamase/mL (kindly supplied by University of Bristol) were used to neutralise CPT. The β-lactamase neutralised CPT, up to a concentration of 75 mg/L. CPT was added to nutrient agar plates at x1, x2, x4 and x8, the MIC of the strains tested in studies on emergence of resistance.

Strains

Three strains of Enterobacteriaceae were used. CPT MICs were performed with a fixed concentration of 4 mg/L avibactam. The strains were *E. coli* SMD 35576 CTX-M-producer (CPT MIC 0.08 mg/L); *Enterobacter cloacae* SMD 42424 AmpC-producer (CPT MIC 1.8 mg/L); and *K. pneumoniae* SMD 42421 KPC-producer (CPT MIC 3.6 mg/L).

The *E. cloacae* and *K. pneumoniae* strains were kindly supplied by Dr R Jones, JMI laboratories, North Liberty, Iowa, USA.

Pharmacokinetics

CPT was dosed 12-hourly or 8-hourly for 48 h for human dosing simulations. Drug concentrations of CPT were determined by HPLC. In addition, a series of avibactam dose fractionation studies were performed comparing avibactam 600 mg 12-hourly to 1200 mg 24-hourly, and 600 mg 8-hourly to 1800 mg 24-hourly. Continuous infusions at 0.5, 1, 2, 4, 6, 8 and 10 mg/L and dose fractionation of 24 h avibactam AUC of 1, 2, 18, 24, 36, 72 and 108 mg/L.h was also performed.

Antibacterial effects (ABE)

Experiments were performed at an initial inoculum of CFU/10^6 mL, prepared as previously described. Samples were taken throughout the simulation period for detection of viable counts. Bacteria were quantified by spiral plater (Don Whitley Spiral Systems, Shipley, West Yorkshire, England). The minimum level of detection is 10^2 CFU/mL.

Pharmacodynamics and measurement of ABE

The ABE of CPT was calculated by determining the log change in viable counts at 24 h (d24), and d24 related to pharmacokinetic parameters; that is, AUC (mg/L.h), C_{max} (mg/L), T>1 mg/L, T>2 mg/L, T>4 mg/L (%) using a sigmoid E_{max} model.

Results

- The dose simulations used in these analyses are shown in **Table 1**
  - two data sets were analysed, the first comprising 20 experiments with CTX-M-producing *E. coli*, 35 experiments involving AmpC-hyperproducing *E. cloacae* and 20 experiments involving KPC-producing *K. pneumoniae*
  - a second data set was analysed which included 35 experiments with CTX-M-producing *E. coli*, 42 experiments with AmpC-hyperproducing *E. cloacae*, and 36 experiments with KPC-producing *K. pneumoniae*
- The relationships between AUC, C_{max}, T>1 mg/L, T>2 mg/L, T>4 mg/L avibactam are shown in **Figures 1–3**
  - for AUC, the R^2 were in the range 0.672–0.978; C_{max}, 0.746–0.845; and T>1, 2 or 4 mg/L, R^2 all ≤0.4
- The relationships between AUC, C_{max}, T>1 mg/L, T>2 mg/L and T>4 mg/L are shown in **Figures 4, 5 and 6**
  - for AUC, the R^2 were in the range 0.5–0.74; C_{max}, 0.616–0.748; and T>1 mg/L, 2 mg/L or 4 mg/L, R^2 all ≤0.54

Table 1. Dose regimens of ceftaroline plus avibactam used in analysis

Ceftaroline	Avibactam	*E. coli* CTX-M-producer	*E. cloacae* Amp C-producer	*K. pneumoniae* KPC-producer
Data set A				
400 mg 8-hourly	600 mg 8-hourly	✓	✓	✓
600 mg 12-hourly	600 mg 12-hourly	✓	✓	✓
600 mg 8-hourly	600 mg 8-hourly	✓	✓	✓
600 mg 12-hourly	1200 mg 24-hourly	✓	✓	✓
600 mg 8-hourly	1800 mg 24-hourly	✓	✓	✓
Data set B				
600 mg 8-hourly	0			
	Cl 0.5 mg/L	✓	✓	✓
	Cl 1 mg/L	✓	✓	✓
	Cl 2 mg/L	✓	✓	✓
	Cl 4 mg/L	✓	✓	✓
	Cl 6 mg/L	✓	✓	✓
	Cl 8 mg/L	✓	✓	✓
	Cl 10 mg/L	✓	✓	✓
	AUC_{24} 1.2 mg/L.h 24-hourly or 8-hourly	✓	✓	×
	AUC_{24} 18 mg.L 24-hourly or 8-hourly	×	×	✓
	AUC_{24} 24 mg.L 24-hourly or 8-hourly	✓	✓	×
	AUC_{24} 36 mg/L.h 24-hourly or 8-hourly	✓	×	✓
	AUC_{24} 72 mg/L.h 24-hourly or 8-hourly	✓	×	✓
	AUC_{24} 108 mg/L 24-hourly or 8-hourly	×	×	✓

Figure 1. Relationship between AUC and d24 for *E. coli* CTX-M (solid line; R^2 0.804); *E. cloacae* AmpC (dashed line; R^2 0.978); and *K. pneumoniae* KPC (dashed/dotted line; R^2 0.672)

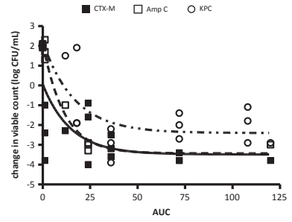


Figure 2. Relationship between C_{max} and d24 for *E. coli* CTX-M (solid line; R^2 0.845); *E. cloacae* AmpC (dashed line; R^2 0.769); and *K. pneumoniae* KPC (dashed/dotted line; R^2 0.746)

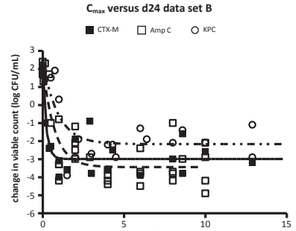


Figure 3. Relationship between A) T>1 mg/L, B) T>2 mg/L, C) T>4 mg/L and d24 for *E. coli* CTX-M (solid line); *E. cloacae* AmpC (dashed line); and *K. pneumoniae* KPC (dashed/dotted line)

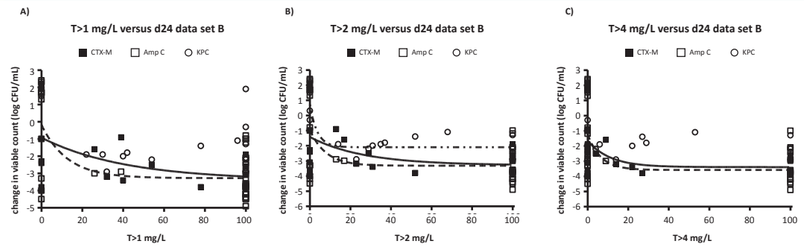


Figure 4. Relationship between AUC and d24 for *E. coli* CTX-M (solid line; R^2 0.5); *E. cloacae* AmpC (dashed line; R^2 0.74); and *K. pneumoniae* KPC (dashed/dotted line; R^2 0.54)

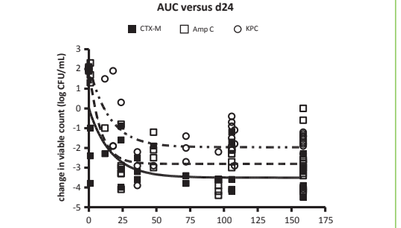


Figure 5. Relationship between C_{max} and d24 for *E. coli* CTX-M (solid line; R^2 0.748); *E. cloacae* AmpC (dashed line; R^2 0.696); and *K. pneumoniae* KPC (dashed/dotted line; R^2 0.612)

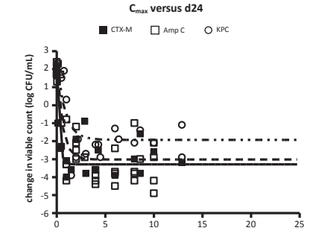
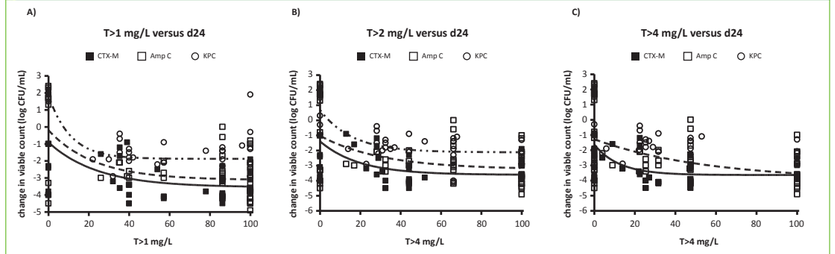


Figure 6. Relationship between A) T>1 mg/L, B) T>2 mg/L, C) T>4 mg/L and d24 for *E. coli* CTX-M (solid line); *E. cloacae* AmpC (dashed line); and *K. pneumoniae* KPC (dashed/dotted line)



Conclusions

- **Using this extensive data set on three species of Enterobacteriaceae with diverse β-lactamase enzyme production, C_{max} and AUC were most closely related to log clearance of pathogen at 24 h**
- **For some strains in some data sets, AUC was more closely related than C_{max}, and for other strains, the relationship was reversed**
- **The data in this study show that T>threshold (%), though related to log drop in viable count, was not as closely related as C_{max} or AUC**

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