Pharmacokinetics-Pharmacodynamics of Tazobactam in Combination with Ceftolozane in an *In Vitro* Model

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ABSTRACT

Objectives: Despite β-lactamase inhibitors being available for clinical use for nearly 30 years, no study has robustly discriminated the PK-PD determinants of efficacy for these agents.
Methods: Dose fractionation studies were designed to 1) determine the exposure measure most predictive of tazobactam (TAZ) efficacy in combination with ceftolozane (TOL); 2) determine the magnitude of this measure necessary for antibacterial efficacy; and 3) determine the impact of β-lactamase expression on the magnitude of the exposure measure necessary for efficacy in a PK-PD *in vitro* infection model. The challenge organism panel was an isogenic CTXM-15-elaborating *Escherichia coli* triplet set, which differed in enzyme expression (low, moderate, high). The hydrolytic activity for the low-, moderate-, and high-β-lactamase-producing triplet set was 36, 120, and 580, respectively. The same daily TAZ dose was fractionated on an every 6, 8, 12, and 24 h schedule, while the TOL schedule was every 8 h regardless of TAZ schedule. Change in CFU relative to TAZ maximum concentration (C_{max}), area under the concentration-time curve (AUC), and % Time above threshold (%T>Threshold) concentration were fit using a Hill-type model. The threshold concentration was determined by evaluation of the dispersion of data along the exposure axis and r^2 value optimization for the relationship between $\log_{10}$ CFU at 24 h and %T>Threshold. Susceptibility testing was conducted in accordance with CLSI guidelines.
Results: While the MIC of TOL/TAZ was 0.25 mg/L, regardless of the amount of β-lactamase produced, the MIC of TOL alone was 4, 16, and 64 mg/L for low-, moderate-, and high-β-lactamase-producing strains, respectively. The TAZ exposure measure that was most closely associated with efficacy was %T>Threshold (r^2=0.938), regardless of enzyme expression (Figure 3). The threshold concentration was 0.05 mg/L for low- and moderate-β-lactamase expression constructs, while it was 0.25 mg/L for the high-β-lactamase expression construct. The magnitude of TAZ %T>threshold associated with net bacterial stasis and a 1- and 2-$\log_{10}$ CFU reduction at 24 h was approximately 35, 50, and 70%, respectively.
Conclusions: These data provide an initial target TAZ concentration-time profile and paradigm to optimize TAZ dosing when combined with TOL.

INTRODUCTION

- Due to the rapidly rising clinical concern of ß-lactamas-producing Enterbacteriaceae, many new ß-lactamase inhibitor/ß-lactam combinations are under clinical development. ß-lactamase inhibitors have been effectively used clinically for nearly 30 years with little data describing the pharmacokinetic-pharmacodynamic (PK-PD) determinants of efficacy for these agents.
- *In vitro* infection models can be utilized to describe the potential efficacy of microbial agents by determining the PK-PD measure predictive of efficacy as well as the magnitude of the PK-PD measure necessary for efficacy.
- In these studies, we utilized an *in vitro* infection model to determine the PK-PD driver of tazobactam (TAZ) in combination with ceftolozane (TOL) using three isogenic CTX-M-15 producing *Escherichia coli* strains.

OBJECTIVES

- Using an *in vitro* infection model, the objectives of these analyses were the following:
  - To identify the PK-PD measure that best predicts TAZ efficacy in combination with TOL versus three isogenic *E. coli* strains, each producing differing levels of CTX-M-15;
  - To determine if increasing ß-lactamase transcription levels have an impact upon the PK-PD measure associated with efficacy; and
  - To determine the magnitude of PK-PD measure associated with net bacterial stasis, and a 1- to 2-$\log_{10}$ reduction in bacterial counts at 24 h for each of the isogenic strains.

METHODS

Test Compounds

- Ceftolozane and tazobactam were provided by Cubist Pharmaceuticals (Lexington, MA).

Strains

- A clinical wild-type TOL-susceptible *E. coli* strain was transformed using recombinant vectors carrying the $bla_{CTX\text{-}M\text{-}15}$ gene and varying promoter regions (to provide different transcription levels of $bla_{CTX\text{-}M\text{-}15}$).
- Three of these isogenic *E.coli* strains, each producing differing levels of CTX-M-15 (low, moderate, and high), were utilized in these studies.

Susceptibility Testing, Transcription Levels, and Hydrolytic Activity

- Strain susceptibilities were performed over a 2 day period using CLSI guidelines and cation-adusted Mueller Hinton broth (MHB) for TOL alone and in combination with TAZ (4 mg/L).
- Quantitative real-time PCR (qRT-PCR) assays were performed using an endogenous reference gene (*rspL*) comparing the expression levels of $bla_{CTX\text{-}M\text{-}15}$ to that of the *E. coli* strain demonstrating the lowest CTX-M-15 production based on minimum inhibitory concentration (MIC) results and hydrolysis assays.
- The hydrolytic activity of the CTX-M-15 enzyme produced by each strain was measured by observing the changes in absorbance due to the opening of the TOL ß-lactam ring using a UV/visible spectrophotometer over a 2 minute interval. The degree of hydrolytic activity was calculated using the following formula:

$$\text{Hydrolytic activity} = \frac{\Delta\ \text{Absorbance/minute}}{\text{protein concentration (mg/L)}} \times \text{(-1,000 (factor)}$$

One-compartment PK-PD *In vitro* Model

- The model consisted of a central infection compartment containing MHB, the challenge isolate, and magnetic stir bar to ensure homogeneity of the drug(s). The entire unit was placed within an incubator set at 35° C.
- Drug-free medium was pumped in and out of the central compartment via computer controlled peristaltic pumps and computer-controlled syringe pumps were utilized to simulate the 2.5 and 1 h half-lives of TOL and TAZ, respectively.
- An initial inoculum of 1.0 x 10^6 colony forming units (CFU)/mL was utilized for each challenge isolate.
- Samples were taken for the enumeration of bacterial density and quantification of drug concentrations throughout the duration of the study. All bacterial cultures were washed twice with normal saline, serially diluted, and cultured on drug free agar for CFU determination.

Dose Range Studies

- Dose range studies were conducted using each strain to determine dose-response relationships. TOL (125-1000 mg) and TAZ (31.25-2000 mg) doses were administered alone and in combination every 8 h (results not shown).

Dose Fractionation Studies

- TOL and TAZ regimens were selected using the results of the dose range studies. Total daily TAZ exposure, as measured by free-drug 24 h area under the concentration-time curve ($AUC_{0\text{-}24}$), was held constant, but fractionated into doses administered every 6, 8, 12, and 24 h. TOL doses were administered every 8 h. Studies were performed in duplicate.

Analytical Method

- Samples for both TOL and TAZ were assayed by LC/MS/MS (Waters, Milford, MA) with lower limits of quantification of 0.1 mg/L.

METHODS

PK-PD Analysis

- Data from the dose fractionation studies were evaluated using Hill-type models and non-linear least squares regression and weighted using the inverse of the estimated measurement variance.
- Relationships between change in $\log_{10}$ CFU/mL at 24 h and $AUC_{0\text{-}24}$, maximum concentration (C_{max}), and percent time above threshold (%T>Threshold) were evaluated.
- Thresholds were identified through an iterative process where concentrations of 0.01 to 1 mg/L were evaluated and discriminated based upon optimization of r^2 values for the relationship between change in $\log_{10}$ CFU at 24 h and %T>Threshold.

RESULTS

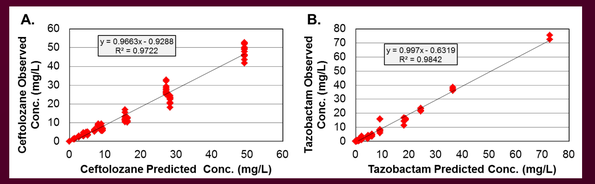
- The MIC values for TOL alone were determined to be 4, 16, and 64 mg/L, respectively, for all three strains (**Table 1.**) Each of the three strains had a MIC value of 0.25 mg/L when tested using TOL in the presence of TAZ at 4mg/L.
- The hydrolytic activity rates and relative transcription levels increased with the MIC accordingly.
- The predicted TOL and TAZ PK profiles were well-simulated resulting in r^2 values of 0.972 and 0.984 for TOL and TAZ, respectively (**Figure 1.**)

Table 1. Susceptibility testing, hydrolytic activity rates, and transcription levels of $bla_{CTX\text{-}M\text{-}15}$

E. coli strain	TOL alone	TOL + TAZ 4 mg/L	Hydrolytic activity[a]	qRT-PCR[b]
Control	0.25	0.25	-3	ND
Low	4	0.25	36	2.7
Moderate	16	0.25	120	22.6
High	64	0.25	580	120.3

a. Hydrolytic activity rates expressed as TOL hydrolyzed per minute per mg of protein.
b. Expression of $bla_{CTX\text{-}M\text{-}15}$ relative to the strain demonstrating the lowest CTX-M-15 production based upon MIC results and hydrolysis assays.

Figure 1. The relationships between observed and targeted TOL (A) and TAZ (B) concentrations



- The dose fractionation studies showed TAZ efficacy increased with dosing frequency (**Figure 2.**). As evidenced by the relationships between change in $\log_{10}$ CFU and PK-PD measures in **Figure 3**, %T>Threshold was the PK-PD measure associated with efficacy (r^2=0.938).
- The magnitude of TAZ exposure (and hence, threshold) required for net bacterial stasis and a 1- to 2-$\log_{10}$ reduction increased as the magnitude of ß-lactamase expression increased (**Figure 4**).

RESULTS

Figure 2. Averaged dose fractionation results showing the effect of each active regimen in comparison to the no-treatment controls for the low (A), moderate (B), and high (C) CTX-M-15 producing *E. coli* strains

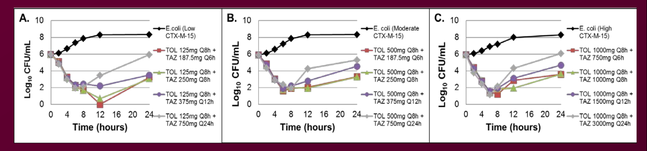


Figure 3. Relationships between the change in $\log_{10}$ CFU and TAZ exposure measures at 24 h of therapy for the low, moderate, and high CTX-M-15 producing *E. coli* strains

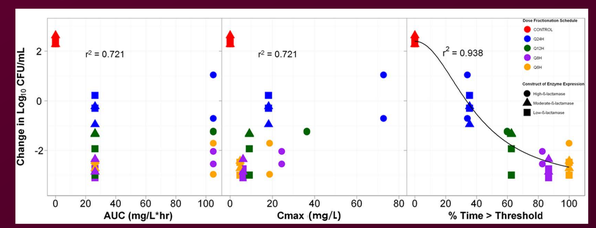
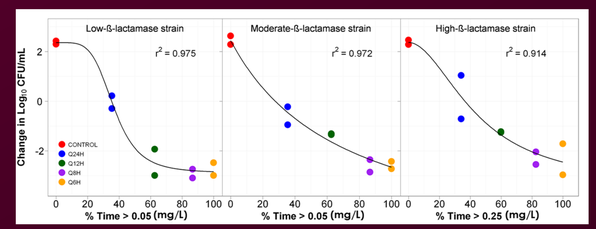


Figure 4. Relationships between the change in $\log_{10}$ CFU and TAZ %T>Threshold at 24 h of therapy for low, moderate, and high CTX-M-15 producing *E. coli* strains



CONCLUSIONS

- %T>Threshold was identified as the TAZ PK-PD measure associated with efficacy for these isogenic CTX-M-15 producing *E.coli* strains.
- The threshold concentration was greater for the high CTX-M-15 producing (0.25 mg/L) compared to the low and moderate CTX-M-15 producing constructs (0.05 mg/L).
- The magnitude of the %T>Threshold associated with net bacterial stasis, and a 1- and a 2-$\log_{10}$ CFU reduction at 24 h was approximately 35, 50, and 70, respectively, regardless of enzyme transcription level.

DISCLOSURES

Funding for ceftolozane/tazobactam studies provided by Cubist Pharmaceuticals, Inc.