Pharmacodynamics of Ceftolozane Combined with Tazobactam against *Enterobacteriaceae* in a Neutropenic Mouse Thigh Model

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Ceftolozane is a new broad-spectrum cephalosporin and is combined with tazobactam to broaden the activity of ceftolozane against strains producing extended-spectrum beta-lactamases (ESBLs). We determined the pharmacodynamics (PD) of the combination in the neutropenic mouse thigh model to determine the optimal exposure of tazobactam. Treatment of CD-1 neutropenic mice was started 2 h after infection with ceftolozane every 2 h (q2h) alone or in combination with tazobactam at different dosing frequencies for 24 h, and the number of CFU in the thighs was determined before and after treatment. The maximum effect model was fit to the dose-response and the pharmacokinetic/PD index (PDI)-response to determine the PDI values for ceftolozane alone and ceftolozane in combination with tazobactam resulting in a static effect and a 1-log kill. The effect of tazobactam was dependent on the percentage of time that the free drug concentration remained above the concentration threshold (percent $fT_{>C_T}$), whereby dosing q2h was more efficacious than dosing every 8 h (q8h), reducing the tazobactam daily dose by a factor 6.9 to 59.0 ($n = 3$ strains) to obtain a static effect. Using R^2 as an indicator of the best fit of the percent $fT_{>C_T}$ -response relationships, the concentration threshold best correlating with the response varied from 0.5 to 2 mg/liter, depending on the strain. A similar result was obtained when the q2h and q8h regimens were analyzed. For all isolates tested, the mean $fT_{>C_T}$ for 0.5 mg/liter tazobactam was 28.2% (range, 17.5 to 45.8%) and 44.4% (range, 26.6 to 54.7%) for a static effect and a 1-log kill, respectively, at ceftolozane exposures that produced a ceftolozane concentration of 4 mg/liter (a concentration greater than the MIC) for 33.9 to 63.3% of a 24-h period under steady-state pharmacokinetic conditions. The main PDI that correlated with the effect of tazobactam was the $fT_{>C_T}$ achieved with a C_T of 0.5 mg/liter tazobactam.

Resistance to beta-lactam antibiotics and the rapid spread thereof are major concerns and are likely to remain so. In particular, infections caused by beta-lactamase-producing bacteria present a therapeutic dilemma when the beta-lactam antibiotic alone is ineffective (1–4). Ceftolozane is a novel cephalosporin that has activity against *Pseudomonas aeruginosa* and other Gram-negative bacteria as well as certain Gram-positive bacteria. However, the drug is susceptible to degradation by extended-spectrum beta-lactamases (ESBLs). *In vitro* studies have shown that elevated MICs of ceftolozane against resistant strains of the *Enterobacteriaceae* or *Pseudomonas* strains were restored to the levels for susceptible strains in the presence of the beta-lactamase inhibitor tazobactam (5, 6). This increase of drug efficacy after addition of tazobactam has been confirmed in a murine thigh infection model (7). However, the optimal tazobactam dosing regimen for the treatment of infections caused by ESBL producers still needs to be verified.

Little is known about the antimicrobial effect of ceftolozane *in vivo* when tazobactam is coadministered using different doses or dosing frequencies and about the pharmacodynamic (PD) effects of tazobactam and the consequences for therapy. It has been shown that for the beta-lactamase inhibitor relebactam (MK7655), the total daily dose (TDD) rather than the dosing frequency or the time above a concentration threshold (C_T) was linked to the effect (8), whereas for avibactam, another beta-lactamase inhibitor, C_T appeared to drive efficacy (7, 9, 10). Thus, beta-lactam inhibitors in combination with a beta-lactam agent do not necessarily have the same pharmacokinetic (PK)/PD driver as a predictor of the antibacterial effect. In a study by VanScoy and colleagues in which a hollow-fiber model and simulation of hu-

man exposures of ceftolozane-tazobactam combinations were used, C_T seemed to be the primary driver as well (10). Therefore, in this study, we explored the exposure-response relationship (ERR) over 24 h of treatment with ceftolozane alone and in combination with tazobactam using several clinical *Klebsiella pneumoniae* and *Escherichia coli* isolates and the well-established neutropenic mouse thigh infection model (11, 12). These strains were chosen as they displayed different underlying resistance mechanisms and exhibited various MICs for ceftolozane and diverse effects in response to the inhibitor.

The exposure-response relationship of tazobactam was investigated using different dosing regimens and dosing intervals of tazobactam combined with a fixed ceftolozane dose that alone showed no efficacy against *K. pneumoniae* and *E. coli*, an approach that we have used before (8).

MATERIALS AND METHODS

Setting and animals. All animal experiments were carried out in the Central Animal Facility (Centraal Dierenlab) at Radboud University Medical

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TABLE 1 Phenotypes (MICs), beta-lactamase genotypes, and pharmacodynamic characteristics of the isolates used in the experiments^a

Species and strain	Resistance summary (ESBL expressed)	MIC (mg/liter)								Static % $fT_{>MIC}$ for TOL
		CAZ	TOL	TOL-TAZ at 0.25 mg/liter	TOL-TAZ at 0.5 mg/liter	TOL-TAZ at 1 mg/liter	TOL-TAZ at 2 mg/liter	TOL-TAZ at 4 mg/liter	TOL-TAZ at 8 mg/liter	
<i>K. pneumoniae</i>										
17	CTX-M15, SHV-1, OXA-1	>128	>64	>64	>64	>64	>64	4	2	No effect
49	TEM-1, SHV-5, OXA-1	>128	32	64	32	16	16	32	16	No effect
58	TEM-84, SHV-11	64	8	8	4	1	1	0.25	0.25	62.6
74	CTX-M1	4	32	8	4	1	1	0.5	0.5	47.7
<i>E. coli</i>										
3	CTX-M15, TEM-84	32	>64	32	16	4	1	0.5	0.25	No effect
15	CTX-M15	64	>64	32	8	4	1	0.5	0.5	No effect
41	CTX-M2, TEM-1	8	32	32	32	16	8	2	1	No effect
82	CTX-M14	2	16	8	8	1	1	0.5	0.25	23.0
11100J	CTX-M15, Pc hybrid 1	4	8	0.25	0.25	0.25	0.25	0.25	0.25	5.6
10771J	CTX-M15, Pc strong + P2	16	32	0.5	0.25	0.25	0.25	0.25	0.25	38.8

^a CAZ, ceftazidime; TOL, ceftolozane; TAZ, tazobactam; Pc hybrid 1, gene expression “slow” or “weak” CTX-M producer; Pc strong + P2, gene expression “hyper” CTX-M producer.

Centre, Nijmegen, The Netherlands, and were conducted in accordance with the recommendations of the European Community (directive 2010/63/EU, 22 September 2010). The studies were approved by the Animal Welfare Committee of Radboud University (no. RU-DEC 2011-102 and RU-DEC 2012-231). Outbred female CD-1 mice obtained from Charles River, The Netherlands (weight, 18 to 22 g at arrival), were used in the experiments. The animals were allowed to acclimatize for at least 5 days upon arrival and received food and water *ad libitum*. The mice were rendered neutropenic by intraperitoneal (i.p.) injection of cyclophosphamide on days −4 (when cyclophosphamide was administered at 150 mg/kg of body weight) and −1 (when cyclophosphamide was administered at 100 mg/kg) prior to infection (13).

Drugs. Ceftolozane and tazobactam were supplied by Cubist Pharmaceuticals and were reconstituted in normal sterile saline (0.9% NaCl) according to the manufacturer's instructions to concentrations of 200 mg/ml for ceftolozane and 100 mg/ml for tazobactam. The solutions were stored at −80°C until use. The drug dilutions needed to obtain the concentrations used in the experiments were prepared with normal saline. The levels of protein binding, obtained from the results of our previous studies (14), were taken to be 5.1% (standard error [SE], 0.85%) for ceftolozane and 25.1% (SE, 2.45%) for tazobactam.

Strains. Eight clinical isolates (four *K. pneumoniae* isolates and four *E. coli* isolates) and two *E. coli* strains provided by JMI Laboratories were used in the experiments. The strains exhibited various ceftolozane MICs and various levels of beta-lactamase expression (Table 1). MIC determinations and full checkerboard experiments were conducted at least in duplicate in accordance with ISO-compliant methods (15).

Infection and dosing. Mice were infected with approximately 10^6 bacteria by inoculating each thigh with a different strain. Treatment with either ceftolozane alone or ceftolozane in combination with tazobactam or a sham control treatment (saline) by the intraperitoneal route was started 2 h after infection and every 2 h (q2h) thereafter for 24 h. In the monotherapy studies, ceftolozane was given q2h in 2-fold increasing doses ranging from 2 to 64 or 4 to 128 mg/kg, corresponding to the percentage of a 24-h period that the drug concentration exceeded the MIC under steady-state PK conditions ($fT_{>MIC}$) of 0% for the lowest dose and 77.7% for the highest dose. For the dose fractionation studies of tazobactam, varying the dose and dose interval, tazobactam was administered q2h

or q8h or as a single dose q24h with a q2h ceftolozane dosing regimen corresponding to approximately 2 and/or 1 $\log_{10}$ growth above the static effect for ceftolozane alone. The efficacy of tazobactam was determined by measurement of the change in the number of CFU relative to the number of CFU for the controls. The pharmacodynamic indices of tazobactam in the presence of ceftolozane at a fixed dose of were determined for three strains: strains 15, 58, and 10771J. The fixed dose used was selected on basis of the response to ceftolozane monotherapy. In addition, six strains were tested for further validation using a fixed interval of tazobactam dosing with increasing doses. All dosing regimens were given to groups of at least two animals.

Sampling and analysis. At time zero (2 h after inoculation), 2 mice were humanely killed to determine the initial inoculum just before treatment. All other animals were killed at 24 h unless the welfare of the animals necessitated earlier termination, in accordance with animal welfare regulations. Excised thighs were transferred to a precooled 12-ml plastic tube (item no. 163275; Greiner) containing 2 ml phosphate-buffered saline and were homogenized using a T 25 Ultra-Turrax homogenizer. A 10-fold dilution series was prepared, and three aliquots of 10 μ l of each dilution were plated on chromID ESB (bioMérieux) plates. On the following day, the colonies were counted and the number of CFU was calculated. The drug effect was determined as the difference between the $\log_{10}$ number of CFU at 24 h and the $\log_{10}$ number of CFU at time zero (mean value for the control group) and is expressed as the change in the $\log_{10}$ number of CFU.

Pharmacodynamic analysis. Free drug concentrations were used in all calculations. Exposures of unbound ceftolozane and tazobactam were determined using MicLab (version 2.36) software (Medimatics, Maastricht, The Netherlands) and the pharmacokinetic parameter estimates obtained from our previous detailed pharmacokinetic studies in infected neutropenic mice (14). Of note, no drug interaction between ceftolozane and tazobactam was observed in that study. To determine the PK/PD index (PDI) values for ceftolozane alone and ceftolozane in combination with tazobactam that resulted in a net static effect or a 1-log reduction in the organism burden over 24 h, the maximum effect model (or linear regression, if applicable) was fitted to the dose and PDI responses using Prism (version 6.0) software (GraphPad Inc., La Jolla, CA). For the fit of the data obtained with the different dosing regimens obtained in the same experiment, the maximum and minimum effects were constrained to

FIG 1 Examples of exposure-response relationship between the $\log_{10}$ daily dose of ceftolozane (TOL) dosed every 2 h and the change in the $\log_{10}$ number of CFU ($\Delta\log\text{cfu}$) in mouse thighs for two strains, *E. coli* 82 (ceftolozane MIC, 16 mg/liter) (left) and *K. pneumoniae* 58 (ceftolozane MIC, 8 mg/liter) (right). The percent $fT_{>\text{MIC}}$ corresponding to the static doses is 62.6% (left) and 22.9% (right), respectively.

shared estimates. For tazobactam, the percentage of the dosing interval that the free drug concentration remained above the concentration threshold (percent $fT_{>C_T}$), resulting in a static effect or a 1-log kill, was determined with concentrations of 0.125, 0.25, 0.5, 1, 2, 4, and 8 mg/liter.

RESULTS

Pharmacodynamic effects of ceftolozane alone. *In vitro*, declining MICs of ceftolozane were observed in the presence of increasing tazobactam concentrations. Table 1 shows the MIC of ceftolozane alone and in the presence of 0.25 to 8 mg/liter tazobactam for the 10 strains used. Ceftazidime susceptibility is presented as a reference. At a tazobactam concentration of 4 mg/liter, 9 of 10 strains showed MICs of 4 mg/liter or lower, with the exception being *K. pneumoniae* 49.

In vivo, the pharmacodynamic profile of ceftolozane alone was determined for each of the 10 strains. The percent $fT_{>\text{MIC}}$ that demonstrated a static effect is also displayed in Table 1 for each strain. No static effect of ceftolozane alone was attained for half the strains even at the highest dose given q2h. Figure 1 shows examples of the dose-response relationships of ceftolozane alone for 2 of the 10 strains. Ceftolozane alone was very effective *in vivo* against *E. coli* strain 11100J, for which the $fT_{>\text{MIC}}$ for a static effect was 5.6%; therefore, *E. coli* strain 11100J was excluded from any further experiments with tazobactam.

Dose fractionation studies to determine the PK/PD index of tazobactam that best predicts efficacy. Strains *E. coli* 10771J and 15 and *K. pneumoniae* 58 were chosen to initially evaluate the exposure-response relationships of tazobactam combined with ceftolozane. Total daily doses (TDD) of tazobactam varying from 24 to 1,536 mg/kg as well as frequencies of administration of 12

times daily (q2h) and 3 times daily (q8h) were evaluated. Tazobactam dosing was chosen in such a way that the total daily dose was the same for the q2h and q8h experiments for each strain. The exposure-response curves for *E. coli* 10771J obtained with the q2h and q8h dosing regimens are shown as an example in Fig. 2. The results indicate that tazobactam administration q2h was more efficacious than administration q8h, suggesting that the percent $fT_{>C_T}$ is the pharmacodynamic index that best correlates with tazobactam efficacy. Table 2 shows the percent $fT_{>C_T}$ for three *E. coli* strains achieved with a threshold concentration of 1 mg/liter tazobactam. The reduction in the tazobactam daily dose required to obtain a static effect with the combination was a factor of 6.9 for strain 58, 13.5 for strain 15, and 59.0 for strain 10771J.

More dosing regimens were explored in dose fractionation studies with strain *E. coli* 10771J and *K. pneumoniae* 58. Figure 3a and b illustrate the relationship between exposure and effect for various PDIs for strain *E. coli* 10771J and *K. pneumoniae* 58. The best correlation with a PK/PD index value for tazobactam appeared to be the percent $fT_{>C_T}$.

Determination of C_T for tazobactam. To determine which C_T best correlated with efficacy, we used several approaches. The first was to determine the relationship between exposure and efficacy for a range of threshold levels and to decide by visual inspection which looked best. Upon visual inspection of the graphs of the percent $fT_{>C_T}$ against the change in the log number of CFU (concentration thresholds, 0.125, 0.25, 0.5, 1, 2, 4, and 8 mg/liter), 0.5 mg/liter appeared to be the best threshold concentration. To quantify these relationships, the R^2 value for each of the plots was plotted against the threshold concentration, and an example is

FIG 2 Example of exposure-response/dose fractionation for *E. coli* strain 10771J (ceftolozane MIC, 32 mg/liter) obtained with tazobactam (TAZ) regimens administered q2h (left) and q8h (right). Ceftolozane was administered at 8 mg/kg q2h.

TABLE 2 Doses required for stasis and percent $fT_{>C_T}$ of tazobactam obtained with the q2h and q8h dosing regimens^a

Strain	MIC (mg/liter)	TOL dose (mg/kg) q2h	% $fT_{>MIC}$ for TOL	q2h dosing regimen		q8h dosing regimen	
				TDD (mg/kg) of TAZ required for stasis	% $fT_{>C_T}$ TAZ	TDD (mg/kg) of TAZ required for stasis	% $fT_{>C_T}$ TAZ
10771J	32	8	0	51.2	23.8	3020.9	19
58	8	8	18.2	933.8	61.0	No	
58	8	32	48.7	223.8	42.7	1549.2	16.9
15	>64	32	0	84.7	30.3	1141.8	15.9

^a The C_T was 0.5 mg/liter tazobactam. TOL, ceftolozane; TAZ, tazobactam; TDD, total daily dose; no, no static effect reached.

shown in Fig. 4. The best fits ranged from 0.5 to 2 mg/liter; for *E. coli* 10771J, *E. coli* 15, and *K. pneumoniae* 58, these were 0.5 mg/liter ($R^2 = 0.97$), 1 mg/liter ($R^2 = 0.81$), and 2 mg/liter ($R^2 = 0.78$), respectively. The mean $fT_{>C_T}$ obtained for these strains using these threshold values was 23.2% (standard deviation [SD], 1.9%).

The second approach was to compare the q2h and q8h dosing regimens. If C_T is indeed the PK/PD index that best predicts efficacy, there should be a C_T where the percent $fT_{>C_T}$ -response relationships of the q2h and q8h regimens do not significantly differ from each other. We therefore compared the best-fit curves of the percent $fT_{>C_T}$ obtained with a range of C_T s for the q2h and q8h dosing regimens and determined whether the curves were or were not significantly different for each C_T . Figure 5 shows an example of the results, obtained with *E. coli* 10771J, indicating that for this strain the threshold was 0.5 mg/liter. For the other two strains, the

thresholds were 1 mg/liter (*E. coli* 15) and 4 mg/liter (*K. pneumoniae* 58).

Estimation of percent $fT_{>C_T}$. The efficacy of tazobactam against six other strains (strains 3, 17, 41, 49, 74, and 82) was further studied primarily to further quantify the relationship between the tazobactam concentration and effect. Because the dose fractionation studies had indicated that the PK/PD index that best correlated with outcome was the percent $fT_{>C_T}$, tazobactam doses were administered at a fixed dose and at the same time as ceftolozane. The values of the percent $fT_{>C_T}$ of tazobactam at four different thresholds for bacterial stasis and a 1-log₁₀ kill are shown in Table 3 for all nine strains.

The exposure (percent $fT_{>C_T}$)-response relationship (ERR) for the seven tazobactam threshold concentrations (between 0.125 and 8 mg/liter) was also determined for each of the six responding isolates. Subsequently, the R^2 value of each of the seven ERRs was

FIG 3 Dose-response relationships in tazobactam dose fractionation experiments. The thighs of mice were infected with *K. pneumoniae* 58 (a) and *E. coli* strain 10771J (b). Fixed doses of ceftolozane of 32 mg/kg for *K. pneumoniae* 58 and 8 mg/kg for *E. coli* strain 10771J were coadministered q2h. fAUC, area under the concentration-time curve for free drug; fC_{max} , maximum concentration in plasma for free drug.

FIG 4 Relationship between R^2 values for the percent $fT_{>C_T}$ against the change in the log number of CFU (for concentration thresholds of 0.125, 0.25, 0.5, 1, 2, 4, and 8 mg/liter) and the tazobactam concentration threshold for *E. coli* 10771J (ceftolozane MIC, 32 mg/liter). Ceftolozane dosing regimen, 8 mg/kg q2h.

plotted against the corresponding C_T and the C_T corresponding with the best fit (the C_T with the highest R^2 value; Fig. 4) determined. Using this value as the tazobactam threshold for the percent $fT_{>C_T}$ for each strain, the result was a mean value of 24.2% (SD, 4.3%). Using a C_T of 0.5 mg/liter tazobactam, $fT_{>C_T}$ values of 17.5 to 45.8% were required for a static effect and $fT_{>C_T}$ values of 26.6 to 54.7% were required for a 1- $\log_{10}$ kill. For two of the four *K. pneumoniae* strains (strains 17 and 49), a clear exposure effect was not observed with the dosing regimens used.

DISCUSSION

In this study, we used various tazobactam doses combined with a suboptimal ceftolozane therapeutic dose in order to determine the PK/PD of tazobactam in the neutropenic mouse thigh infection model. When tazobactam was combined with ceftolozane, tazobactam increased the activity of ceftolozane *in vitro* as well as *in vivo* against otherwise ceftolozane-resistant strains. Similar to the findings obtained in a study with avibactam (9), the percent $fT_{>C_T}$ was the PD index that was the most predictive of the exposure-response relationship of tazobactam. There did not appear to be a relationship between the maximum concentration in plasma (C_{max}) for tazobactam, the area under the concentration-time curve, or the total daily dose (TDD) and efficacy. A similar observation was made by VanScoy and colleagues in studies with *in vitro* infection models (10, 16). In the dose fractionation experiments performed in the present study, the best fit of exposure-response was obtained using the percent $fT_{>C_T}$ obtained with tazobactam at 0.5 to 2 mg/liter.

Craig and Andes reported that the percent $fT_{>MIC}$ was the primary index driving the efficacy of ceftolozane, with a mean value for stasis of $25.2\% \pm 2.8\%$ being found for four wild-type strains of the *Enterobacteriaceae* and four susceptible wild-type *P. aeruginosa* strains, which was lower than the mean value found for other cephalosporins (17). In the present study, we found an average $fT_{>MIC}$ of 35.2% for the five strains for which a static effect could be observed during ceftolozane monotherapy, and this value was

FIG 5 Relationship of percent $fT_{>C_T}$ for tazobactam against the change in the log number of CFU in the thighs of mice infected with *E. coli* strain 10771J obtained with the q2h and q8h regimens. GraphPad Prism was used to create the graphs. The top and bottom of each curve was constrained by shared value for all data sets. Fixed doses of ceftolozane (8 mg/kg) were coadministered q2h.

closer to that observed for other cephalosporins. When one of the five strains was excluded from our calculations because of its very low $fT_{>MIC}$ value of 5.6%, which was derived from the low level of production of beta-lactamase, the static effect increased to the extent that it was in agreement with the effects demonstrated by the other cephalosporins (17). Thus, we found no evidence that lower exposures were required for ceftolozane than for other cephalosporins, even though all the strains that we tested did have a resistance mechanism.

VanScoy et al. proposed that the tazobactam C_T achieved with $0.5 \times MIC$ of ceftolozane-tazobactam at 4 mg/liter for the percent $fT_{>C_T}$ of tazobactam was the best predictor of the efficacy of the combination ceftolozane-tazobactam in their hollow-fiber model studies (16). The authors based this relationship on pooled data from the percent $fT_{>C_T}$ and the change in the $\log_{10}$ number of CFU from the baseline at 24 h, which gave a high R^2 value. Using the same approach for the pooled data set, we determined the fits for $0.25 \times$, $0.5 \times$, and $1 \times MIC$ (data not shown). The fit for $0.5 \times MIC$ did indeed provide the best results, in that the measure of error for the percent $fT_{>C_T}$ was the lowest. However, if the R^2 value is taken as a reference for the best fit when examining different thresholds for single strains, a value for fit for C_T even better than that obtained with $0.5 \times MIC$ was found and was up to four 2-fold dilutions for one *K. pneumoniae* strain (for this isolate, $0.5 \times MIC$ ceftolozane-tazobactam at 4 mg/liter gave a C_T value of 0.125 and an R^2 value of 0.7625, but for C_T values ranging from 0.125 to 8 mg/liter, the concentration that gave the best R^2 value was 2 mg/liter, which gave an R^2 value of 0.7765 and which was thus four 2-fold dilutions higher than the $0.5 \times MIC$ value). For one *E. coli* strain, the MIC value and not $0.5 \times$ the MIC value was slightly better. However, the differences were marginal. Since the $0.5 \times MIC$ values were systematically lower than the C_T values, the mean percent $fT_{>C_T}$ for the $0.5 \times MIC$ value that resulted in a static effect was somewhat higher (39.0% versus 28.2%).

Another approach to validate the C_T could be to assume that an $fT_{>MIC}$ of 30 to 40% is required for the efficacy of ceftolozane, as was observed during monotherapy in this study and as has been described for other cephalosporins, and to use the MIC from the *in vitro* checkerboards to find the concentration of tazobactam needed for the corresponding MIC of ceftolozane to match an $fT_{>MIC}$ of 30 to 40% and subsequently determine the percent $fT_{>C_T}$ for that specific concentration. This approach was also used for relebactam (18). The mean percent $fT_{>C_T}$ for tazobactam needed for stasis determined using this method was 23.5% (SD, 14.8%).

Although tazobactam improved the efficacy of ceftolozane in both species, in general, *E. coli* strains were more responsive to the ceftolozane-tazobactam combination than the *K. pneumoniae* strains. Tazobactam exposures might have been the limiting factor in the case of the two nonresponding *K. pneumoniae* strains, and higher exposures may be required. This was evidenced by the results for *K. pneumoniae* 58. In most experiments, we used ceftolozane exposures that provided an $fT_{>MIC}$ of 0 to 18.2% and that were thus ineffective as monotherapy to identify the PK/PD index for tazobactam. For *K. pneumoniae* 58, we required a higher exposure of ceftolozane alone that provided an $fT_{>MIC}$ of 48.7%, which was still below the value associated with bacterial stasis for this strain, because we thought the dosing regimen used for ceftolozane ($18.2\% fT_{>MIC}$) might be a limiting factor since stasis was only reached with the highest exposure tazobactam regimen. The

TABLE 3 Percent $fT_{>C_T}$ of tazobactam for stasis and a 1-log kill for 9 strains for C_T s of 0.25, 0.5, 1, and 4 mg/liter tazobactam^a

Strain	MIC (mg/liter)	TOL dose (mg/kg)	% $fT_{>MIC}$ for TOL	TDD (mg/kg) of TAZ required for stasis	% $fT_{>C_T}$ required for stasis for TAZ at:				TDD (mg/kg) of TAZ required for a 1-log kill	% $fT_{>C_T}$ required for a 1-log kill for TAZ at:				
					0.25 mg/liter	0.5 mg/liter	1 mg/liter	4 mg/liter		0.25 mg/liter	0.5 mg/liter	1 mg/liter	4 mg/liter	
<i>K. pneumoniae</i>														
17	>64	32	0	No										
49	32	32	18.2	No	51.6	42.7	33.7	15.6	543.7	63.0	54.1	45.2	27.3	
58	8	32	48.7	223.8	26.5	17.5	8.2	0	248.2	53.0	44.0	35.0	16.9	
74	32	32	18.2	31.4										
<i>E. coli</i>														
3	>64	32	0	32.0	26.7	17.8	8.5	0	434.8	60.1	51.2	42.3	24.3	
15	>64	32	0	84.7	39.2	30.3	21.1	2.8	331.0	56.6	47.7	38.8	20.7	
41	32	32	18.2	284.0	54.7	45.8	36.8	18.7	570.7	63.6	54.7	45.8	27.9	
82	16	16	18.2	36.3	28.3	19.4	10.1	0	63.7	35.5	26.6	17.4	0.0	
107711	32	8	0	51.2	32.7	23.8	14.5	0	101.0	41.4	32.5	23.4	5.1	
Mean					37.1	28.2	17.7	5.3		53.3	44.4	35.4	17.5	
SD					11.8	11.9	12	8.2		10.9	10.9	11.1	11.0	

^a TOL, ceftolozane; TAZ, tazobactam; TDD, total daily dose; no, no static effect reached. All dosing regimens were administered q8h.

approximately 2.5 times higher level of ceftolozane exposure resulted in a net 2-log₁₀-CFU reduction at 24 h, and a level of tazobactam exposure about 1.5 times less was needed for a net bacterial stasis using, in this case, a concentration threshold of 1 mg/liter tazobactam. The concentration of tazobactam required to achieve stasis is far higher than that which one would expect for this isolate, for which the MIC for ceftolozane alone was relatively low (8 mg/liter) and for which the MIC of ceftolozane in combination with 4 mg/liter tazobactam was 0.25 mg/liter, and is far higher than the concentration required to achieve stasis for the other isolates, suggesting that other factors, such as resistance determinants, gene expression, and factors like growth rate *in vivo*, might play a role in the high concentration required to achieve stasis. This may also have been the cause of the greater variability in CFU counts at levels around the static dose level than at other levels of exposure. Another explanation could be that there were diverse levels of transcription of beta-lactamases, given the differential levels of expression of beta-lactamase enzymes in bacteria (19, 20), and the presence of various beta-lactamase variants may indicate the reason for the differential C₇s of tazobactam (16).

Although it was not the purpose of this study, we briefly explored whether the pharmacodynamic targets achieved with the 500-mg dose of tazobactam now used clinically in combination with 1 g ceftolozane every 8 h and an infusion duration of 1 h would match the pharmacodynamic targets found in this study. Using data from a recently developed population model of tazobactam (21) and the percent $fT_{>C_T}$ obtained with tazobactam at 0.5 mg/liter corresponding to pharmacodynamic targets of 28.2% for stasis and 44.4% for a 1-log kill, the probability of target attainment was 1 and over 0.99, respectively, thus supporting the dose currently used.

In conclusion, the effect of tazobactam was dependent on the dosing frequency; a decreased effect was observed with a decreased dosing frequency. The main PK/PD index that correlated with an effect was the time above the threshold concentration of tazobactam, and the magnitude was 28.2% and 44.4% for a static effect and a 1-log kill, respectively. The magnitude of the effect of tazobactam was also dependent, in part, on the level of ceftolozane exposure.

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