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In vitro pharmacodynamic evaluation of ceftolozane/tazobactam against β-lactamase-producing *Escherichia coli* in a hollow-fibre infection model

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ABSTRACT

The proliferation of multidrug-resistant Gram-negative pathogens has been exacerbated by a lack of novel agents in current development by pharmaceutical companies. Ceftolozane/tazobactam was recently approved by the FDA for the treatment of complicated intra-abdominal infections and complicated urinary tract infections. In the present study, the activity of ceftolozane/tazobactam against four isogenic *Escherichia coli* strains was investigated in a hollow-fibre infection model simulating various clinical dosing regimens. The four investigational *E. coli* strains included #2805 (no β-lactamase), #2890 (AmpC β-lactamase), #2842 (CMY-10 β-lactamase) and #2807 (CTX-M-15 β-lactamase). Each strain was exposed to regimens simulating 1 g ceftolozane, 2 g ceftolozane, 1 g ceftolozane/0.5 g tazobactam, and 2 g ceftolozane/1 g tazobactam utilising a starting inoculum of ca. 10^6 CFU/mL. Whereas 1 g of ceftolozane eradicated strains #2805 and #2842 without subsequent regrowth, 1 g ceftolozane/0.5 g tazobactam was required to eradicate strain #2890. For strain #2890, ceftolozane monotherapy led to bacterial growth on plates impregnated with 20 mg/L ceftolozane by 24 h, whilst combination treatment with tazobactam completely suppressed the development of ceftolozane resistance. In contrast, none of the regimens, including 2 g ceftolozane/1 g tazobactam, were able to entirely suppress bacterial growth in strain #2807, with bacterial counts exceeding 10^8 CFU/mL by 48 h and ceftolozane-resistant populations being amplified after 24 h. Thus, the combination of ceftolozane and tazobactam achieved bactericidal activity followed by sustained killing over 10 days for three of four isogenic *E. coli* strains. Ceftolozane/tazobactam is a promising new agent to counter multidrug-resistant Gram-negative bacteria.

1. Introduction

The escalating incidence of multidrug-resistant organisms coupled with the marked decline in antimicrobial drug development efforts has severely limited the therapeutic armamentarium against numerous problematic pathogens [1]. Over the past decade, global attention has been directed at β-lactamase-producing *Escherichia coli*, highlighted by its classification from the Infectious Diseases Society of America (IDSA) as a priority drug-resistant microbe for which new therapies are urgently required [2]. Recent drug discovery initiatives targeting novel cephalosporin analogues with potent antipseudomonal activity led to the identification of ceftolozane [3]. However, the efficacy of ceftolozane against Gram-negative infections is compromised in the presence of carbapenemases, metallo-β-lactamases and extended-spectrum β-lactamases (ESBLs) [4,5], thus directing efforts to develop ceftolozane in combination with the β-lactamase inhibitor tazobactam, which enables its spectrum of activity to be enhanced against a range of multidrug-resistant Enterobacteriaceae. This novel combination has been subsequently designated ceftolozane/tazobactam and was recently approved by the US Food and Drug Administration (FDA) for the treatment of complicated urinary tract infections and complicated intra-abdominal infections [6].

Judicious use of antimicrobials is an important strategy to preserve antimicrobial efficacy and to combat rising rates of multidrug resistance worldwide. This in part involves efforts to optimise dosage regimens in order to enhance activity and minimise the development of resistance. Consequently, pharmacokinetic (PK) and

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pharmacodynamic (PD) principles are important factors to consider in establishing the relationship between the time course of drug concentrations/exposure and drug effect at the desired site of action. Previously, we demonstrated that the ceftolozane susceptibility of β-lactamase-producing *E. coli* markedly increased in the presence of increasing tazobactam concentrations [7]. The aim of this study was to evaluate the pharmacodynamics of clinical regimens of ceftolozane alone and in combination with tazobactam against four β-lactamase-producing *E. coli* strains in an in vitro hollow-fibre infection model (HFIM), whereby the input and elimination of ceftolozane and tazobactam are simulated according to human PK parameters [8,9] in order to accurately represent drug exposure over time.

2. Materials and methods

2.1. Antimicrobial agents and bacterial strains

Bacterial strain descriptions, the antimicrobial agents utilised and minimum inhibitory concentration (MIC) determinations were the same as previously reported [7]. Briefly, four isogenic strains of *E. coli* differing in their expression of a single defined β-lactamase were investigated (Merck & Co., Lexington, MA) (Table 1). To construct these strains, the β-lactamase open reading frame (ORF) was assembled using published GenBank sequences inserted into a modified pBR322 (GenBank accession no. **J01749**) cloning vector, replacing the native bla_{TEM-1} ORF, and then introduced into the β-lactamase-deficient parent strain, *E. coli* DH10B, to produce strain #2890 [*Pseudomonas aeruginosa ampC* and *ampR* (5′ region), GenBank accession no. **X54719.1**], strain #2842 [*E. coli* K998298 ESBL precursor (bla_{CMY-10}), GenBank accession no. **AF381617.1**] and strain #2807 [*E. coli* strain 405/06 plasmid pKC405 β-lactamase CTX-M-15 ($bla_{CTX-M-15}$) and insertion sequence IS*26* TnpA, GenBank accession no. **GQ274933.1**].

β-Lactamase gene expression in the engineered strains was driven by the native bla_{TEM-1} promoter in the modified pBR322 plasmid to allow for similar levels of β-lactamase expression in each *E. coli* strain. The MICs of ceftolozane alone (MIC_{TOL}) and ceftolozane/tazobactam 4 mg/L (MIC_{TAZ4}) were calculated for each *E. coli* strain. These included: (i) #2805, wild-type, no β-lactamase (MIC_{TOL} = 0.25 mg/L): (ii) #2890 (AmpC; MIC_{TOL} = 4 mg/L, MIC_{TAZ4} = 1 mg/L); (iii) #2842 (CMY-10; MIC_{TOL} = 8 mg/L, MIC_{TAZ4} = 4 mg/L); and (iv) #2807 (CTX-M-15; MIC_{TOL} > 128 mg/L, MIC_{TAZ4} = 8 mg/L). MICs were determined according to standard broth microdilution methods [12]. MICs were not determined in the presence of tazobactam 4 mg/L for wild-type strain #2805 owing to the absence of a β-lactamase enzyme.

2.2. Hollow-fibre infection model

The HFIM has been described previously [13,14]. Investigations were conducted over 10 days (240 h) using a two-compartment system comprised of a sterile central reservoir and a cellulose cartridge (FiberCell Systems Inc., Frederick, MD) with an extracapillary space of 15 mL for bacterial containment. Prior to each experiment, bacterial colonies from an overnight culture on Mueller–Hinton agar (BD, Franklin Lakes, NJ) were re-suspended in Mueller–Hinton broth (BD), were grown to mid-logarithmic phase and were adjusted to obtain a suspension corresponding to a McFarland standard of ca. 0.5. Then, 15 mL of this suspension was diluted to 10^6 CFU/mL and was used to inoculate the HFIM by placement into the extracapillary space. The HFIM contains multiple ports for the input and removal of broth, delivery of antibiotics, and serial collection of bacterial and drug samples.

Fresh broth was continuously pumped into the HFIM to generate the humanised half-life ($t_{1/2}$) of each drug. Each central reservoir and cellulose cartridge was housed in an incubator at 37 °C for the duration of the experiment. Ceftolozane ($t_{1/2}$ = 2.3 h) and tazobactam ($t_{1/2}$ = 1 h) were added into the central reservoir using a previously described approach [9,15]. Desired free-drug plasma concentrations (fC_{max}) of both drugs were targeted according to previously published PK parameters in healthy volunteers [8,9] following administration of four clinical regimens every 8 h, including: (i) 1 g ceftolozane (fC_{max} = 46.1 mg/L); (ii) 2 g ceftolozane (fC_{max} = 100.9 mg/L); (iii) 1 g ceftolozane (fC_{max} = 46.1 mg/L)/0.5 g tazobactam (tazobactam fC_{max} = 21.3 mg/L); and (iv) 2 g ceftolozane (fC_{max} = 100.9 mg/L)/1 g tazobactam (tazobactam fC_{max} = 42.6 mg/L). Free drug concentrations were calculated based on 21% protein binding for ceftolozane [16] and 30% for tazobactam [17,18]. Samples were withdrawn aseptically from the cartridge before the first dose (0 h sample) as well as at 2, 4, 6, 24, 26, 30, 48, 50, 54, 72, 74, 78, 96, 98, 102, 144, 192 and 240 h after the initial dose for determination of total bacterial counts. Bacteria were quantified on drug-free horse blood agar with 5% sheep's blood (BD) as previously described [19].

The concentrations of ceftolozane and tazobactam for each of the four regimens were validated from samples withdrawn from the central reservoir during a separate experiment that was performed prior to the addition of bacteria. Over the course of 48 h, PK samples were collected and were immediately frozen at –80 °C. A validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) method was then used to analyse all of the PK samples for ceftolozane and tazobactam concentrations (MicroConstants Inc., San Diego, CA). The lower limit of quantification in broth was 0.25 mg/L for ceftolozane and 0.1 mg/L for tazobactam. The precision and accuracy of the assays for ceftolozane and tazobactam were within acceptable limits (≤10%) for both agents.

2.3. Population analysis profiles (PAPs)

PAPs were completed in order to quantify the emergence of ceftolozane-resistant populations following exposure to each regimen. Ceftolozane/tazobactam breakpoints according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for Enterobacteriaceae were utilised (susceptible, ≤1 mg/L; resistant, >1 mg/L) [11]. Accordingly, 50 μL samples at 0, 24, 48, 72, 96, 144, 192 and 240 h were plated on Mueller–Hinton agar impregnated with ceftolozane (without tazobactam) at concentrations of 0.5, 1, 2, 3, 4, 6, 8, 10 and 20 mg/L and were then incubated at 37 °C for ca. 48 h. Baseline PAPs at time 0 h were only performed on the untreated growth control for each strain. For assessment of viable counts and PAPs, bacterial counts were determined using a laser bacterial colony counter (ProtoCOL v.2.05.02; Synbiosis, Cambridge, UK) and were expressed as $\log_{10}$ CFU/mL. Bactericidal activity (99.9% kill) was associated with a ≥3.0 $\log_{10}$ CFU/mL reduction in bacterial density compared with the initial inoculum.

Table 1
Bacterial strains utilised for hollow-fibre infection model experiments, including description of β-lactamase enzymes and ceftolozane minimum inhibitory concentrations (MICs) in the presence and absence of 4 mg/L tazobactam (TAZ).

Strain	β-Lactamase		Ceftolozane MIC (mg/L)	
	Type	Ambler class [10]	TAZ 0	TAZ 4
#2805	None	—	0.25	—
#2890	AmpC	C	4	1
#2842	CMY-10	C	8	4
#2807	CTX-M-15	A	>128	8

European Committee on Antimicrobial Susceptibility Testing (EUCAST) ceftolozane/tazobactam breakpoints for Enterobacteriaceae: susceptible, ≤1 mg/L; resistant, >1 mg/L [11].

3. Results

The total change in bacterial burden and PAPs illustrating the growth of ceftolozane-resistant populations in the presence of ceftolozane (0.5–20 mg/L) are presented for strains #2805, #2890, #2842 and #2807 in Fig. 1.