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Determination of the Pharmacodynamic Activity of Clinically Achievable Tigecycline Serum Concentrations Against Clinical Isolates of *Escherichia coli* with Extended-Spectrum (ESBLs), AmpC β-Lactamases and Reduced Susceptibility to Carbapenems Using an *In Vitro* Model

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REVISED ABSTRACT

Background: *Escherichia coli* harbouring ESBLs, AmpC β-lactamases and reduced susceptibility to carbapenems (CRS) are increasing worldwide. This study assessed the *in vitro* pharmacodynamic activity of tigecycline against *E. coli* with ESBLs, AmpCs and CRS.

Methods: Nine *E. coli* were studied including three ESBL, three AmpC-producing, and three isolates demonstrating CRS (ertapenem MIC, ≥0.12mg/L). The pharmacodynamic model was inoculated with organisms at 1x10^6 CFU/mL and tigecycline dosed once every 24h to simulate the fC_{max} (free peak serum concentration) and $t_{1/2}$ (serum half-life) obtained after standard 100mg intravenous every 24h dosing (fC_{max}, 0.15mg/L; $t_{1/2}$ 42h). Samples were collected over 48h.

Results: For isolates with tigecycline $fAUC_{24}$/MIC of 2.0 (tigecycline MIC, 0.5 mg/L), tigecycline demonstrated bacteriostatic activity with <1 log_{10} reduction in bacterial growth compared to the initial inoculum at 12, 24, and 48h. Against the two isolates for which the tigecycline $fAUC_{24}$/MIC was 4.0 (tigecycline MIC, 0.25 mg/L), tigecycline demonstrated bacteriostatic activity with ~1.5 log_{10} reduction in bacterial growth compared to the initial inoculum at 12, 24 and 48h. Against the two isolates for which the tigecycline $fAUC_{24}$/MIC was 8 (tigecycline MIC, 0.12 mg/L), tigecycline demonstrated bacteriostatic activity with ~2.0 log_{10} reduction in bacterial growth compared to the initial inoculum at 12, 24 and 48h.

Conclusions: Tigecycline demonstrated ~1-2 log_{10} killing against *E. coli* harbouring ESBLs, AmpC β-lactamases and reduced susceptibility to carbapenems when simulating clinically achievable serum concentrations and represents a potential therapy for infections caused by these isolates.

INTRODUCTION

Escherichia coli continues to be an important pathogen associated with both community-associated and healthcare-associated infections.[1] The spread of resistance to third-generation cephalosporins in *E. coli* has been increasing in both hospital and community settings. Resistance to cephalosporins is often mediated by the production of extended-spectrum β-lactamases (ESBLs), however, it may also be attributed to AmpC cephalosporinases which, in *E. coli*, generally arise by acquisition of a plasmid-mediated AmpC enzyme.[2] *E. coli* with reduced susceptibility to carbapenems (ertapenem MIC, ≥0.12mg/L) have been isolated from urine, blood, wounds as well as from patients with respiratory tract infections.[1] β -lactam-resistant *E. coli* are frequently multidrug-resistant (MDR, resistance to ≥3 different antimicrobial classes) and limit therapeutic options for patients.[3]

Tigecycline (Tygacil™, Wyeth), the first-in-class glycylcycline, was developed to recapture the broad spectrum of activity of the tetracycline class and to treat patients with resistant bacterial infections.[4] Tigecycline's *in vitro* spectrum of activity encompasses aerobic, facultative, and anaerobic gram-positive and gram-negative bacteria, including antimicrobial-resistant and MDR bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* (VRE), and extended-spectrum ß-lactamase (ESBL)-producing *Enterobacteriaceae*.[4] Clinical trials involving patients with complicated skin and skin structure infections and complicated intra-abdominal infections, including patients infected with MRSA, demonstrated that tigecycline was bacteriologically and clinically effective with only mild to moderate gastrointestinal adverse events (i.e., nausea, vomiting, diarrhea).[4] Tigecycline is a promising broad-spectrum parenteral monotherapy for the treatment of patients with gram-positive and gram-negative bacterial infections.

MATERIALS AND METHODS

Bacterial isolates and culture conditions: The isolates used in this study have been well described.[1-3] They were three ESBL-producing *E. coli* (all CTX-M-15), three AmpC-producing isolates (two genotype CMY-2; one ACT-1), and three isolates demonstrating reduced susceptibility to carbapenems (ertapenem MIC, ≥0.12mg/L; all three isolates were also ESBL-producers) [Table 1].[1-3] The ESBL-producing isolates were more frequently MDR than were the AmpC-producing isolates [Table 1]. Tigecycline MICs ranged from 0.12-0.5mg/L.

For the pharmacodynamic studies, logarithmic phase cultures were prepared by initially suspending several colonies in cation-supplemented Mueller Hinton broth (Oxoid, Nepean, Ontario) at a density equivalent to a 0.5 McFarland standard (1x10^8 CFU/mL). This suspension was then diluted 1:100 and 20µL of the diluted suspension was further diluted in 60mL of cation-supplemented Mueller Hinton broth. Following overnight growth at 37°C, suspensions were diluted 1:10 and approximately 60mL of the diluted suspension was added to the *in vitro* pharmacodynamic model. Viable bacterial counts consistently yielded a starting inoculum of approximately 1x10^6 CFU/mL.[5,6]

Antimicrobial susceptibility testing: Tigecycline was obtained as laboratory-grade powder from the manufacturer (Wyeth, New Jersey, USA). Stock solutions of each agent were prepared and dilutions made according to the Clinical and Laboratory Standards Institute (CLSI) M7-A7 method.[7] Following two subcultures from frozen stock, MICs for each of the isolates were determined by the CLSI broth microdilution method.[7] All MICs were performed in triplicate on separate days.

***In vitro* pharmacodynamic model:** Experiments were performed simulating peak free drug concentrations (fC_{max}) and $fAUC_{24}$ of tigecycline achieved in serum after standard intravenous doses in healthy volunteers (tigecycline 100mg [fC_{max} 0.15mg/L]).[4,8] Protein free (f), also known as unbound, serum concentrations were simulated using known protein binding fractions (tigecycline ~80-85%).[4,8] Tigecycline clearance was simulated using a reported serum half-life of 42h.[4,8] The pharmacokinetics of tigecycline were evaluated by dosing using standard doses in the central compartment of the model and sampling (10µL aliquots) from this compartment at 0, 1, 2, 4, 6, 8, 12, 16, 24, 36 and 48h. Tigecycline concentrations were determined in quadruplicate using *Bacillus cereus* as the test organism.[5,6] Specifically, tigecycline concentrations were determined by measuring the diameters of zones of inhibition resulting from plated aliquots and comparing those zone sizes to zones sizes produced by known concentrations from a standard series. The lower and upper detection limits were 0.03mg/L and 32mg/L of tigecycline, respectively; the relationship between zone sizes and MICs was linear over this range of concentrations. Both the inter-day and intra-day variation was <15%. The $fAUC_{24}$ (mg.h/L) for tigecycline was calculated using the trapezoidal rule.[5,6] The $fAUC_{24}$/MIC was calculated for tigecycline against each of the *E. coli* strains studied.

The *in vitro* pharmacodynamic model used in this study has been previously described.[5,6] Logarithmic phase cultures were diluted (1:10) into fresh (<12h aged) cation-supplemented Mueller Hinton broth to achieve a starting inoculum of approximately 1x10^6 CFU/mL. This initial inoculum was introduced into the central compartment (volume; 0.610L) of the *in vitro* pharmacodynamic model and exposed to tigecycline, simulating free (protein unbound) serum concentrations obtained after a standard 100mg intravenous dose. Pharmacodynamic experiments were performed in ambient air at 37°C. At 0, 1, 2, 4, 6, 8, 12, 16, 24, 36 and 48h, samples were removed from the central compartment and viable bacterial counts performed by plating serial 10-fold dilutions onto Tryptic Soy agar with 5% sheep blood. Plates were incubated overnight at 37°C in ambient air. The lowest dilution plated was 0.1mL of undiluted sample and the lowest level of detection was 200 CFU/mL (20 colonies grown from 0.1mL of undiluted sample). Antibiotic carryover was minimized by diluting samples withdrawn from the model. Bacterial inhibition (log_{10} killing) of organisms by tigecycline from the model was assessed at 12, 24 and 48h and compared to the initial inoculum. Changes in CFU/mL at 48h were compared using analysis of variance with Tukey's post hoc test. A P value ≤0.05 was considered significant.

RESULTS

Table 1. Susceptibility profiles of nine isolates of *Escherichia coli* for tigecycline and comparator antimicrobial agents

Isolate	MIC (mg/L)						
	Tigecycline	Ceftriaxone	Cefepime	Ciprofloxacin	Gentamicin	Ertapenem	Piperacillin-Tazobactam
59096-ESBL	0.5	32	4	32	0.25	0.03	1
62188-ESBL	0.5	128	16	32	16	0.06	1
62199-ESBL	0.5	256	16	32	0.5	0.03	8
61550-AmpC	0.5	8	1	1	8	0.03	8
62567-AmpC	0.25	64	1	32	0.5	0.03	8
63370-AmpC	0.12	32	1	0.03	0.25	0.03	4
78967-CRS	0.5	128	4	0.5	0.5	0.12	256
80960-CRS	0.25	128	64	32	1	0.25	4
81687-CRS	0.12	128	64	32	32	0.25	128

ESBL - Extended spectrum β-lactamase; CRS - carbapenem (ertapenem) reduced susceptibility

Table 2. Tigecycline pharmacodynamic parameters achieved in the *in vitro* pharmacodynamic model

Isolate	Tigecycline (mg/L)	$fT_{>MIC}$ h [%]	$fCmax$ /MIC	$fAUC_{24}$ /MIC
59096-ESBL	0.5	0 [0]	0	2.0
62188-ESBL	0.5	0 [0]	0	2.0
62199-ESBL	0.5	0 [0]	0	2.0
61550-AmpC	0.5	0 [0]	0	2.0
62567-AmpC	0.25	0 [0]	0	4.0
63370-AmpC	0.12	14 [58]	1.3	8.0
78967-CRS	0.5	0 [0]	0	2.0
80960-CRS	0.25	0 [0]	0	4.0
81687-CRS	0.12	14 [58]	1.3	8.0

ESBL - Extended spectrum β-lactamase; CRS - carbapenem (ertapenem) reduced susceptibility

Table 3. Tigecycline killing for nine isolates of *Escherichia coli* in a *in vitro* pharmacodynamic model simulating free serum concentrations

Isolate	Log_{10} killing at 12, 24 and 48h[a]		
	12h	24h	48h
59096-ESBL	0.7±0.5	0.9±0.5	0.9±0.6
62188-ESBL	0.7±0.4	0.8±0.5	0.8±0.5
62199-ESBL	0.9±0.4	0.8±0.4	0.7±0.4
61550-AmpC	0.8±0.4	0.7±0.3	0.7±0.4
62567-AmpC	1.3±0.5	1.4±0.5	1.4±0.4
63370-AmpC	2.1±0.5	2.0±0.6	2.1±0.7
78967-CRS	0.6±0.2	0.6±0.3	0.4±0.5
80960-CRS	1.5±0.3	1.6±0.6	1.4±0.6
81687-CRS	2.0±0.6	2.0±0.5	2.1±0.4

[a] - growth reduction relative to initial inoculum
ESBL - Extended spectrum β-lactamase; CRS - carbapenem (ertapenem) reduced susceptibility

PURPOSE

The purpose of this study was to assess the pharmacodynamic activity of clinically achievable tigecycline serum concentrations against clinical isolates of *E. coli* harbouring ESBLs, AmpCs, and isolates with reduced susceptibility to carbapenems using an *in vitro* model.

CONCLUSIONS

1. Tigecycline demonstrated ~1-2 log_{10} killing against *E. coli* harbouring ESBL's, AmpC β-lactamases and reduced susceptibility to carbapenems when simulating clinically achievable serum concentrations.
2. Tigecycline represents a potential therapy for infections caused by MDR ESBL *E. coli*.

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REFERENCES

1. Zhanel GG, DeCorby M, Laing N *et al*. Antimicrobial resistant pathogens in intensive care units across Canada: Results of the Canadian National Intensive Care Unit (CAN-ICU) Study, 2005/2006. Antimicrob Agents Chemother 2008;52:1430-7.
2. Zhanel GG, Karlowsky JA, DeCorby M et al. Prevalence of antimicrobial-resistant pathogens in Canadian hospitals: results of the Canadian ward surveillance study (CANWARD 2007). Can J Infect Dis Med Microbiol 2009;20(Supplement A):9A-19A.
3. Baudry T, Nichol K, DeCorby M *et al*. Comparison of antimicrobial resistance profiles among Extended-Spectrum β-Lactamase producing- and plasmid mediated AmpC β-Lactamase producing *Escherichia coli* from Canadian Intensive Care Units. Antimicrob Agents and Chemother 2008;52:1430-37.
4. Zhanel GG, Karlowsky JA, Rubinstein E *et al*. A review of tigecycline: A novel glycycycline. Exp Rev Antiinfect Ther 2006;4:9-25.
5. Zhanel GG, Baudry P, Vashisht V *et al*. Pharmacodynamic activity of ertapenem versus multi-drug resistant (MDR) genotypically characterized extended spectrum β-lactamase (ESBLS) producing *Escherichia coli* using an *in-vitro* model. J Antimicrob Chemother 2008;61:643-6.
6. Zhanel GG, Roberts D, Laing N, Nichol K, Hoban DJ. Pharmacodynamics of fluoroquinolones vs ciprofloxacin-resistant *Streptococcus pneumoniae*. J Antimicrob Chemother 2002;49:807-12.
7. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard – seventh edition. M7-A7. CLSI, PA, USA, 2006.
8. Rodvold KA, Gotfried MH, Cwik M et al. Serum tissue and body fluid concentrations of tigecycline after a single 100mg dose. J Antimicrob Chemother 2006;58:1221-9.

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