



## In vitro antibacterial activities of tigecycline and comparative agents by time-kill kinetic studies in fresh Mueller-Hinton broth

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### Abstract

Time-kill kinetics performed with tigecycline, in fresh MHB, demonstrated a consistent 1 to 2 log<sub>10</sub> CFU/ml reduction in bacterial counts against the majority of clinically relevant pathogens tested. Although classified as a bacteriostatic agent, tigecycline shows bactericidal activity against select isolates associated with serious infection. In general, vancomycin and imipenem demonstrated bactericidal activity. © 2007 Published by Elsevier Inc.

**Keywords:** Tigecycline; Bacteriostatic; Bactericidal

The management of infections in both the community and hospital settings has become increasingly challenging over the last decade because of the rapid emergence of resistant gram-positive and gram-negative bacteria to a variety of antibiotic classes (Goldstein and Garabedian-Ruffalo, 2002; Jacobs and Johnson, 2003; CDC, 2004). The tetracycline class of antimicrobials is no exception based on the emergence of resistant pathogens with active efflux and ribosomal protection mechanisms of resistance (Connell et al., 2003; Li and Nikaido, 2004). The search for new agents in this class has recently led to the synthesis and development of the glycylycylines, a class specifically positioned to tackle bacteria resistant to earlier generation tetracyclines (Sum and Petersen, 1999). Tigecycline, the first in class glycylycylines, has broad-spectrum in vitro antibacterial activity, which includes tetracycline-resistant organisms. Tigecycline has potency against gram-positive cocci, including methicillin-resistant *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumoniae* and vancomycin-resistant enterococci (Petersen and Bradford, 2005; Petersen et al., 1999). The gram-negative activity of tigecycline extends to many Enterobacteriaceae and some opportunistic pathogens such as *Acinetobacter* spp. and *Stenotrophomonas maltophilia* (Petersen and Bradford, 2005).

Whereas standard in vitro susceptibility testing by MIC measurement provides the clinician with a sense of an antibacterial agent's activity, determination of bactericidal potential by time-kill kinetics is often desirable. The predictive value of time-kill kinetic measurement relative to clinical outcome, however, remains controversial (Pankey and Sabath, 2004). The Clinical and Laboratory Standards Institute (CLSI formerly NCCLS) has approved testing of tigecycline in fresh (<12 hours old) Mueller-Hinton II broth (MHB) as the reference method (CLSI, 2006a, 2006b). This was implemented to stabilize tigecycline, which is oxygen-labile and ensure the reproducibility of testing results. The utilization of the fresh testing methodology (Bradford et al., 2005) resulted in a one to two dilution improvement in in-vitro activity of tigecycline (Petersen and Bradford, 2005) when compared to some of the prior published data. Previous studies have established that tigecycline had both bacteriostatic and bactericidal activity when tested in MHB broth for which the age was not determined (Petersen, P. J., W. J. Weiss, P. Labthavikul, and P. A. Bradford. 38th Intersci. Conf. Antimicrob. Agents Chemother. Abstract. F132 1998). The current study was undertaken to establish the antibacterial activity of tigecycline by time-kill kinetics in fresh MHB to determine if the same trends are seen when fresh media is used.

The clinical isolates used in this evaluation were collected between 2000 and 2004 from various medical

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centers in the U.S. CLSI recommended ATCC quality control strains were also tested for this analysis. The MICs were determined in fresh media as previously described (Petersen and Bradford, 2005) by the broth microdilution method as recommended by the CLSI (CLSI, 2006a, 2006b). Time-kill assays were performed as previously described (Petersen et al., 2004) by the broth macrodilution method, as suggested by CLSI guidelines (NCCLS, 1999). In brief, time-kill curves, were conducted with a final concentration of antimicrobial agent at four times the MIC. Flasks containing 50 ml of MHB with the appropriate antimicrobial agent were inoculated with 50 ml of the test organism in logarithmic growth phase adjusted to the appropriate density. Aliquots were removed, diluted and plated using the WASP II automated spiral plater (Microbiology International, Frederick, MD) at the 0, 2, 4, 6 and 24-hour time point. The plates were incubated for 24 hours and the viable counts determined using the ProtoCOL plate reader (Microbiology International, Frederick, MD). Bactericidal activity was defined as a reduction of 99.9% ( $\geq 3 \log_{10}$ ) of the total count of CFU/ml in the original inoculum (NCCLS, 1999). Bacteriostatic activity was defined as maintenance of or a reduction of less than 99.9% ( $< 3 \log_{10}$ ) of the total count of CFU/ml in the original inoculum.

The antibacterial activities of tigecycline and comparative antimicrobial agents are summarized in Table 1. For the six staphylococcal strains, tigecycline demonstrated a range of reduction in bacterial counts from 0.40 to 3.7  $\log_{10}$  CFU/ml at the 24 hour test period. The reduction in bacterial counts was typically less than 3  $\log_{10}$  CFU/ml, indicative of a bacteriostatic effect, with the exception of one strain of glycopeptide-intermediate *Staphylococcus aureus* (GISA) for which tigecycline exhibited a bactericidal effect with

reduction in counts of 3.7  $\log_{10}$  CFU/ml (Fig. 1A). Tigecycline reduced the bacterial counts of two methicillin-resistant *S. aureus* (MRSA) strains by 1.7 and 1.3  $\log_{10}$  CFU/ml, respectively. Following exposure to tigecycline, the growth of two tetracycline-resistant *S. aureus* strains, one expressing the *tet(K)* efflux pump and one with the ribosomal protection resistance determinant *tet(M)*, were also reduced to a level consistent with a bacteriostatic effect (reduction in count of 1.8 and 0.4  $\log_{10}$  CFU/ml, respectively). Exposure to tigecycline resulted in a bactericidal reduction in viable growth for a penicillin-intermediate and -resistant *Streptococcus pneumoniae* isolate ( $>4.4 \log_{10}$  CFU/ml). Overall, vancomycin had a greater antibacterial effect compared with tigecycline; however, vancomycin provided definitive bactericidal activity ( $\geq 3 \log$  reduction in counts) for only 33% (3 of 9) of the isolates tested.

Tigecycline, with a reduction in bacterial count ranging from 0.95 to 2.8  $\log_{10}$  CFU/ml, demonstrated a pattern of bacteriostatic activity against three of the four *Escherichia coli* strains tested. One *E. coli* strain showed a decrease in viable counts consistent with bactericidal activity (3.3  $\log_{10}$  CFU/ml). Imipenem showed bactericidal activity against two of the *E. coli* strains (range of reduction 4.4 to 4.8  $\log_{10}$  CFU/ml); however, bactericidal activity was not maintained for the duration of the study (24 hours) against the other two strains (Fig. 1B). Tigecycline caused a bacteriostatic reduction (1.9 to 2.0  $\log_{10}$  CFU/ml) against the two *Acinetobacter calcoaceticus/baumannii* complex strains tested. Imipenem showed bactericidal activity against these same strains by the four-hour time-point, however; regrowth was observed by 24 hours (range of reduction 2.2 to 2.6  $\log_{10}$  CFU/ml). Tigecycline showed bacteriostatic activity for one *Klebsiella pneumoniae* strain with a reduction of 0.9  $\log_{10}$

Table 1

Reduction in initial bacterial concentration after 6 and 24 hours of incubation with tigecycline, vancomycin or imipenem at four times the MIC

Organism	6 h/24 h count reduction ( $\log_{10}$ CFU/ml)			MIC ( $\mu\text{g/ml}$ ) <sup>c</sup>		
	Tigecycline	Vancomycin	Imipenem	Tigecycline	Vancomycin	Imipenem
<i>S. aureus</i> ATCC 29213	0.3/1.5	0.8/2.6	ND	0.12	0.5	$\leq 0.06$
<i>S. aureus</i> PT 5381 (MRSA)	0.6/1.7	0.4/2.2	ND	0.12	1	4
<i>S. aureus</i> PT 5644 (MRSA)	0.5/1.3	0.8/4.1	ND	0.12	1	32
<i>S. aureus</i> GC 1079 <i>tet(K)</i>	0.7/1.8	1.5/2.9	ND	0.25	1	$\leq 0.06$
<i>S. aureus</i> GC 6466 <i>tet(M)</i>	0.1/0.40	0.7/2.5	ND	0.06	0.5	2
<i>S. aureus</i> GC 6336 (GISA)	1.7/3.7	0.9/1.8	ND	0.12	4	$\leq 0.06$
<i>E. faecalis</i> ATCC 29212	0.3/0.87	0.8/1.7	ND	0.06	2	0.5
<i>S. pneumoniae</i> ATCC 49619 (Pen-I)	$>4.4/>4.4$	3.6/ $>4.4$	ND	0.015	0.12	$\leq 0.06$
<i>S. pneumoniae</i> GC 1903 (Pen-R)	1.5/ $>4.4$	2.3/ $>4.4$	ND	0.015	0.25	0.25
<i>E. coli</i> ATCC 25922	1.8/2.8	ND <sup>b</sup>	1.8/1.3	0.06	$>64$	0.12
<i>E. coli</i> PT 4300	0.6/2.4	ND	3.0/1.3	0.06	$>64$	0.12
<i>E. coli</i> PT 4836	1.1/3.3	ND	3.9/4.4	0.06	$>64$	0.25
<i>E. coli</i> GC 1073 <i>tet(B)</i>	0.4/0.95	ND	4.3/4.8	0.25	$>64$	0.5
<i>A. calcoaceticus/baumannii</i> PT 7849	2.1/1.9	ND	4.1/2.6	0.12	$>64$	0.25
<i>A. calcoaceticus/baumannii</i> PT 9051	1.3/2.0	ND	3.0/2.2	0.5	$>64$	1
<i>K. pneumoniae</i> PT 4766	1.4/(+) 2.2 <sup>a</sup>	ND	3.9/2.1	0.25	$>64$	0.5
<i>K. pneumoniae</i> PT 4997	0.4/0.90	ND	2.6/4.1	0.25	$>64$	1

<sup>a</sup> Increase in count above initial inoculum.<sup>b</sup> Not determined.<sup>c</sup> Kill-curves performed at 4 times the MIC.

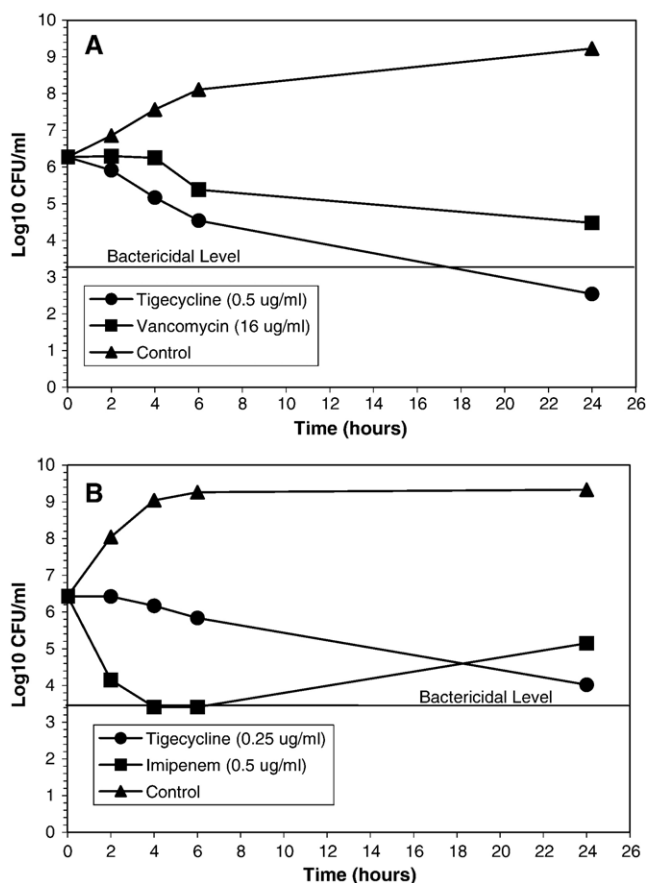


Fig. 1. Time-kill kinetics performed in fresh MHB with (A) tigecycline and vancomycin ( $4 \times \text{MIC}$ ) against *S. aureus* GC 6336 (GISA) and (B) tigecycline and imipenem ( $4 \times \text{MIC}$ ) against *E. coli* PT 4300.

CFU/ml whereas the other *K. pneumoniae* strain demonstrated regrowth at 24 hours following an initial reduction in CFU/ml. Imipenem reduced the initial viable counts to the bactericidal level for the two *K. pneumoniae* isolates tested; however, one of the isolates showed regrowth while the other isolate was maintained at the bactericidal level for the duration of the study (range of reduction 2.1 and 4.1 log<sub>10</sub> CFU/ml).

Establishing whether an agent has bacteriostatic or bactericidal properties provides valuable information on the potential action of antibacterial agents in vitro. However, it is necessary to combine this information with pharmacokinetic/pharmacodynamic data in order to provide more meaningful prediction of efficacy in vivo. Furthermore, bacteriostatic agents (e.g., chloramphenicol, clindamycin and linezolid) may be effective for the treatment of serious infections, such as endocarditis, meningitis, and osteomyelitis (Pankey and Sabath, 2004). In this current in vitro investigation, two of the antibacterial agents (vancomycin and imipenem) generally considered to confer bactericidal activity did not demonstrate bactericidal killing activity against some isolates. The regrowth observed with some strains challenged with imipenem may be due to the labile nature of this compound. When tested in fresh MHB, time-

kill kinetic studies performed with tigecycline provided reliable bacteriostatic and occasional bactericidal activity against clinically important pathogens. The one exception was a strain of *K. pneumoniae* where growth higher than the initial inoculum was observed; however, the mechanism behind this observation has not been determined. Additional in vivo tests may be warranted to fully assess the killing kinetics of tigecycline in the presence of a competent immune system.

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