Postantibiotic Effect of Tigecycline against 14 Gram-Positive Organisms[▽]

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The in vitro postantibiotic effects (PAEs), postantibiotic sub-MIC effects (PA-SMEs), and sub-MIC effects of tigecycline were determined for 14 gram-positive and gram-negative organisms. The pneumococcal, staphylococcal, and enterococcal PAEs were 1.9 to 5.1, 2.9 to 5.7, and 3.9 to 6.1 h, respectively, and those for *Haemophilus influenzae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Acinetobacter baumannii* were 1.1 to 5.0, 1.9 to 2.1, 1.7 to 1.8, 1.0 to 1.7, and 0.7 to 3 h, respectively. The PA-SMEs (four times the MIC) ranged from 6.7 to >11 h for gram-positive organisms and from 2.3 to >11.3 h for gram-negative organisms.

The postantibiotic effect (PAE) is a pharmacodynamic parameter that may be considered in choosing antibiotic dosing regimens. It is defined as the length of time that bacterial growth is suppressed following brief exposure to an antibiotic (6, 7). Odenholt-Tornqvist and coworkers have suggested that, during intermittent dosage regimens, suprainhibitory levels of antibiotic are followed by subinhibitory levels that persist between doses and have hypothesized that persistent subinhibitory levels could extend the PAE. The effect of sub-MICs on growth during the PAE period has been defined as the postantibiotic sub-MIC effect (PA-SME) and represents the time interval that includes the PAE plus the additional time during which growth is suppressed by sub-MICs. In contrast to the PA-SME, the sub-MIC effect (SME) measures the direct effect of subinhibitory levels on cultures which have not been previously exposed to antibiotics (4, 12).

We examined the in vitro PAEs, PA-SMEs, and SMEs of tigecycline, an intravenous broad-spectrum glycylicycline approved for the treatment of complicated skin, soft-tissue, and intra-abdominal infections, against 14 gram-positive and -negative bacteria (1, 3, 8, 9). We studied one strain each of penicillin-susceptible, -intermediate, and -resistant *Streptococcus pneumoniae*; two of methicillin-resistant *Staphylococcus aureus* (one strain vancomycin intermediate and daptomycin resistant); two of *Enterococcus faecalis* (one vancomycin resistant); two of *Haemophilus influenzae* (one β -lactamase negative and ampicillin resistant [BLNAR], the other β -lactamase positive and amoxicillin-clavulanate resistant [BLPACR]); one each of *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*; and two of *Acinetobacter baumannii* (including one multidrug-resistant strain producing OXA-24 and OXA-51 β -lactamases) identified by standard methods (11).

Tigecycline MICs were determined by standard macrodilution procedures (5) with fresh drug powder. The PAE was determined in duplicate by the viable plate count method with freshly prepared Mueller-Hinton broth (Becton Dickinson Co., Sparks, MD) supplemented with 5% lysed horse blood

when testing pneumococci and freshly prepared *Haemophilus* test medium for *H. influenzae* strains. Recent studies have shown that tigecycline is prone to oxidation in broth media (2). In this study, broths were freshly prepared and boiled (5 min) immediately before use. A PAE was induced by exposure to tigecycline at 10 times the MIC for 1 h. An additional PAE was determined by exposure to 2 or 6 times the MIC for 1 h for strains with tigecycline MICs of ≥ 0.12 $\mu\text{g/ml}$.

For PAE testing, tubes containing 5 ml broth with antibiotic were inoculated with approximately 5×10^6 CFU/ml. Inocula were prepared by suspending growth from an overnight blood agar plate in broth. Growth controls with inoculum but no antibiotic were included with each experiment. Inoculated test tubes were placed in a shaking water bath at 35°C for an exposure period of 1 h. At the end of the exposure period, cultures were diluted 1:1,000 in prewarmed broth to remove the antibiotic by dilution. Antibiotic removal was confirmed by comparing the growth curve of a control culture containing no antibiotic to that of another containing tigecycline at 0.001 times the exposure concentration (10 times the MIC).

Viability counts were determined before exposure, immediately after dilution (0 h), and then every 2 h until the turbidity of the tube reached a no. 1 McFarland standard (13).

In cultures designated for PA-SME, the PAE was induced as described above, after exposure to 2, 6, or 10 times the MIC (see above). Following 1:1,000 dilution, cultures were divided into four tubes. To three tubes, tigecycline was added to produce final subinhibitory concentrations of 0.2, 0.3, and 0.4 times the MIC. The fourth tube did not receive antibiotic. Viability counts were determined before exposure, immediately after dilution, and then every 2 h until the culture turbidity reached a no. 1 McFarland standard. Cultures designated for SME were treated the same as for PA-SME testing, except that a PAE was not induced. The formulas used to calculate the PAE, PA-SME, and SME have been previously described (13).

The PA-SME and SME were measured in two separate experiments (done twice, each starting with a new inoculum). For each experiment, viability counts ($\log_{10}$ CFU per milliliter) were plotted against time, and results were expressed as single values (Table 1) and also as the mean of two separate assays.

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TABLE 1. PAEs of tigecycline against 14 strains

Strain description ^b	MIC (μ g/ml)	Exposure ($\times$ MIC) ^c	PAEs (h)	PAEs (h) ^a					
				0.2 $\times$ MIC		0.3 $\times$ MIC		0.4 $\times$ MIC	
				SME ^d	PA-SME ^e	SME	PA-SME	SME	PA-SME
<i>S. pneumoniae</i>									
Pen-S	0.03	10	3.7, 5.0	0, 0.5	5.9, 6.2	0, 0.5	8.8, 9.0	3.0, 0.8	9.0, 9.3
Pen-I	0.06	10	1.9, 2.2	1.9, 2.4	6.1, 6.6	5.7, 7.0	>8.8, >9.0	7.7, 8.0	>8.8, >9.0
Pen-R	0.03	10	4.8, 5.1	0.5, 0.7	6.7, >10.7	0.5, 1.4	>10.2, >10.7	0.7, 1.2	>10.2, >10.7
<i>S. aureus</i>									
Methicillin resistant, vancomycin sensitive	0.12	10	3.2, 3.2	0.2, 0.2	3.6, 4.7	0.2, 0.7	5.2, 5.6	0.9, 1.5	6.7, 7.8
		6	2.9, 3.9	0.2, 0.2	3.7, 5.1	0.2, 0.7	4.9, 6.1	0.9, 1.5	6.9, 7.3
Methicillin resistant, vancomycin intermediate	0.12	10	4.0, 5.7	2.0, 2.4	6.6, 6.8	2.9, 3.6	>10, 9.9	2.9, 4.1	>10, 10.4
		6	4.0, 4.4	2.0, 2.4	6.6, 5.6	2.9, 3.6	10, 9.8	2.9, 4.1	>10, 10.4
<i>E. faecalis</i>									
Vancomycin sensitive	0.06	10	3.9, 4.4	0, 0.4	5.3, 5.5	0.2, 0.4	6.8, 6.8	1.8, 2.4	8.2, 10.4
Vancomycin resistant	0.06	10	6.0, 6.1	0.4, 0.5	7.6, 7.8	0.3, 0.5	10.5, 11.0	0.4, 0.5	11.0, >11.0
<i>H. influenzae</i>									
BLNAR	0.06	10	1.1, 2.2	0.8, 0.9	2.8, 2.9	0.8, 1.5	2.8, 3.5	0.8, 1.5	2.8, 3.7
BLPACR	0.12	10	3.7, 5.0	0, 0.5	3.9, 5.0	0.8, 1.3	5.4, 6.5	1.9, 2.0	7.9, 9.0
		6	3.9, 4.5	0, 0.5	3.9, 4.5	0.8, 1.3	4.7, 6.1	1.9, 2.0	7.7, 9.1
<i>E. coli</i>	0.12	10	2.0, 2.1	0, 0	2.4, 2.7	0, 0.3	2.2, 2.6	0.1, 0.3	3.5, 3.9
		6	1.9, 2.0	0, 0	1.9, 2.3	0, 0.3	2.9, 3.2	0.1, 0.3	3.3, 3.7
<i>K. pneumoniae</i>	0.25	10	1.7, 1.7	0.1, 0.3	1.9, 2.0	0.4, 0.4	2.0, 2.3	0.7, 0.9	2.3, 2.6
		2	1.7, 1.8	0.1, 0.3	1.8, 1.9	0.4, 0.4	2.0, 2.3	0.7, 0.9	2.6, 2.7
<i>E. cloacae</i>	0.5	10	1.2, 1.3	0.2, 0.7	3.6, 3.7	0.4, 1.3	5.0, 5.1	2.7, 2.9	7.2, 7.9
		2	1.0, 1.7	0.2, 0.7	2.9, 3.1	0.4, 1.3	4.9, 5.2	2.7, 2.9	6.9, 8.0
<i>A. baumannii</i>	0.12	10	3.0, 2.9	0.3, 0.9	6.7, 7.2	1.5, 1.9	>11.2, >11.3	>11.2, >11.3	>11.2, >11.3
		6	1.8, 2.2	0.3, 0.9	5.2, 6.0	1.5, 1.9	10.7, >11.3	>11.2, >11.3	>11.2, 11.3
<i>A. baumannii</i> (multidrug resistant)	2.0	10	1.3, 1.4	0.4, 0.5	1.5, 1.6	0.7, 0.7	2.2, 3.0	1.9, 2.0	3.8, 4.3
		2	0.7, 1.0	0.4, 0.5	1.2, 1.7	0.7, 0.7	1.5, 2.1	1.9, 2.0	3.0, 3.5

^a The values shown were obtained in two separate experiments.

^b Pen-S, penicillin susceptible; Pen- I, penicillin intermediate; Pen-R, penicillin resistant; Quin-R, quinolone resistant.

^c Strains were exposed to 10, 6, or 2 times the MIC of tigecycline (see text) for 1 h at 35°C. The drug was removed by 1:1,000 dilution.

^d Strains not previously exposed to tigecycline.

^e Strains previously exposed to tigecycline.

As shown in Table 1, the lengths of the PAEs from gram-positive and -negative strains varied from 1.9 to 6.1 h and from 0.7 to 5.0 h, respectively. PAEs determined following exposure to 2 or 6 times the MIC were either the same or slightly lower than those determined at 10 times the MIC.

For the three pneumococci, the mean PAE was 3.8 h, ranging from 1.9 to 5.1 h. The PAEs for the penicillin-intermediate strain were lower (1.9 to 2.2 h) than those for the sensitive and resistant strains (3.7 to 5.1 h). Pneumococcal PA-SMEs were longer than the PAE and the SME. PA-SMEs at 0.4 times the MIC were >8.8 h. The PAEs for staphylococci were 2.9 to 5.7 h, with a mean of 3.9 h. At 0.4 times the MIC, the PA-SMEs ranged from 6.7 to >10.0 h. For *E. faecalis*, the tigecycline PAEs were 3.9 to 6.1 h, with a mean of 5.1 h. The PAEs for the vancomycin-sensitive strain (3.9 to 4.4 h) were lower than that for the resistant strain (6.0 to 6.1 h).

Haemophilus PAEs ranged from 1.1 to 5.0 h, with a mean of 3.4 h. PAEs and PA-SMEs were lower for the BLNAR strain

than for the BLPACR strain. The BLNAR strain had a mean PAE of 1.6 h, and the PA-SMEs at 0.4 times the MIC ranged from 2.8 to 3.7 h and were slightly higher than the sum of the PAE and the SME. However, the BLPACR strain's mean PAE was 4.3 h and at 0.4 times the MIC, the PA-SMEs ranged from 7.7 to 9.1 h.

For *E. coli*, the tigecycline PAEs ranged from 1.9 to 2.1 h, with a mean of 2.0 h. PA-SMEs at 0.4 times the MIC ranged from 3.3 to 3.9 h. For *K. pneumoniae*, the PAEs ranged from 1.7 to 1.8 h, with a mean of 1.7 h. For *E. cloacae*, the PAEs ranged from 1.0 to 1.7 h, with a mean of 1.3 h. The PA-SMEs at 0.4 times the MIC ranged from 6.9 to 8.0 h. The *A. baumannii* PAEs ranged from 0.7 to 3.0 h, with a mean of 1.8 h; the PAEs and PA-SMEs were shortest for the multiresistant strain. The mean PAE for the multiresistant strain was 1.1 h, compared to 2.5 h for the sensitive strain. At 0.4 times the MIC, the PA-SMEs for the multiresistant strain ranged from 3.0 to 4.3 h, compared to >11.2 h for the sensitive strain. The PA-SMEs for

the multidrug resistant strain were only slightly higher than the sum of the PAE and the SME.

The tigecycline MICs in this study were similar to those described in a recent study (9). Tigecycline is generally regarded to produce long PAEs. Projan reported tigecycline PAEs against *S. aureus* and *E. coli* of >3 h and 1.8 to 2.9 h, respectively (14). In another study, Lefort et al. measured tigecycline PAEs against one *E. faecium* and two *E. faecalis* strains and found PAEs of 2.5 to 3.3 h and 1 to 4.5 h, respectively (10). Mean tigecycline PAEs against *E. coli*, *K. pneumoniae*, and *E. cloacae* of 2 to 5 h were reported (15). The PAEs of the present study are similar to those of these earlier studies and extend those finding with additional strains and the study of tigecycline PA-SMEs and SMEs.

van Ogtrop et al. determined tigecycline PAEs in neutropenic mouse thighs against *S. pneumoniae* and *E. coli* and found PAEs of 8.9 and 4.9 h, respectively (16). Comparison of these in vivo PAEs to those in the present study (Table 1) indicates that they correspond to the PA-SMEs, not the PAEs. This difference can be partly explained by differing techniques, as well as persisting subinhibitory levels of tigecycline that may remain at the site of infection in mice, resulting in an increase in the in vivo PAE. In vivo PAEs may be longer than those measured in vitro because of persisting SMEs, which may be difficult to separate from the true in vivo PAE in an animal model. In vivo PAEs usually correspond to the PA-SME, especially if the antibiotic's half-life is prolonged (4, 6).

The PA-SMEs found in this study were generally longer than the sum of the PAE and the SME for most of the strains tested, with two exceptions. The PA-SMEs roughly approximated (*K. pneumoniae*) or were slightly higher than (BLNAR *H. influenzae* and *A. baumannii*) the sum of the PAEs and the SMEs. This suggests that, for these isolates, subinhibitory concentrations of tigecycline did little or nothing to extend the PAE. The PAEs for the intermediately penicillin-resistant pneumococcus strain were shorter than those for the penicillin-resistant and -sensitive strains. Similarly, the PAEs for the vancomycin-sensitive *E. faecalis* strain were lower than those for the vancomycin-resistant strain. The reason for these differences is unclear but may be related to differences in the rates of killing of these isolates by tigecycline. This hypothesis requires confirmation by the examination of more strains.

Tigecycline is intravenously administered twice daily (initial dose of 100 mg followed by 50 mg every 12 h) for the treatment of infections (Tygacil homepage [http://www.wyeth.com/hcp/tygacil]). It reaches a maximum concentration in serum of approximately 0.6 µg/ml after 60 min and is extensively distributed into tissues. The extended PAE and PA-SME demonstrated in this study, in combination with tigecycline's long half-life (~40 h), support the currently recommended dosage regimen.

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