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In Vitro Activity of Tigecycline against *Staphylococcus epidermidis* Growing in an Adherent-Cell Biofilm Model

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The activity of tigecycline against *Staphylococcus epidermidis* growing in an in vitro adherent-cell biofilm model was determined. Tigecycline minimum bactericidal concentrations (MBCs) ranged from 1 to 8 μg/ml for *S. epidermidis* growing in a biofilm of adherent cells, compared to MBCs of 0.12 to >32 μg/ml for freely growing cells. The killing activity of tigecycline against the adherent bacteria was at least fourfold better than that of vancomycin and daptomycin.

Staphylococcus epidermidis is a leading cause of medical-device-related infections, especially in immunocompromised patients. The treatment of these infections is further complicated by the emergence of multiresistant strains. The ability of *S. epidermidis* to form biofilms on smooth surfaces is believed to contribute significantly to the pathogenesis of these infections. Biofilms are notoriously difficult to eradicate and are often resistant to systemic antibiotic therapy. Numerous studies have reported *S. epidermidis* biofilm resistance to many antimicrobial agents in vitro and in vivo (2, 4, 5, 9, 10, 15, 16, 17).

Tigecycline (formerly GAR-936), a 9-glycylamido derivative of minocycline, is currently in phase III clinical trials. Tigecycline is a broad-spectrum antibacterial agent possessing excellent antimicrobial activity against most gram-positive pathogens, including *S. epidermidis* (1, 7, 13, 14). The goal of this study was to assess the antimicrobial activity of tigecycline against adherent *S. epidermidis* in an in vitro biofilm model.

(This work was presented in part previously [P. Labthavikul and P. A. Bradford, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 525, 2000].)

Clinical isolates of *S. epidermidis* were screened for the ability to produce slime by a method described by Christensen et. al (3). The amount of slime production was scored, and the strains were categorized as strong, moderate, or weak slime producers or non-slime producers. The MICs and minimum bactericidal concentrations (MBCs) of tigecycline and the other antimicrobial agents against adherent *S. epidermidis* were determined as described previously (15, 16), with modifications. Bacterial suspensions (100 μl) at a density of 10^6 CFU/ml were made in phosphate-buffered saline supplemented with 0.25% glucose and were incubated in 96-well plates for 24 h at 37°C, without shaking, to allow the bacteria to attach to the surface. Nonadherent bacteria were removed by gentle washing two times with phosphate-buffered saline with a liquid-handling system (Cetus, Emeryville, Calif.). Serial twofold dilutions (100 μl per well) of antimicrobial agents in Mueller-Hinton broth (MHB II) were added to wells containing adherent cells. The plates were then incubated at 37°C for another 24 h. The MIC^{adh} was defined as the lowest concentration of antibiotic at which there was no observable bacterial growth in the wells containing adherent microcolonies. After the MIC^{adh}s were determined, the MHB II containing antibiotic was removed and replaced with 100 μl of antibiotic-free MHB II; this was followed by incubation for another 20 to 24 h at 37°C. The MBC^{adh} was defined as the lowest concentration of antibiotic at which there was no bacterial growth following removal of the drug. All experiments with adherent cells were performed in duplicate. The MICs and MBCs of tigecycline and other antimicrobial agents for planktonic (freely growing) *S. epidermidis* were determined by broth microdilution assay as recommended by the NCCLS (11, 12) simultaneously with the adherent-cell experiments. To assess the possibility that the inoculum in the MIC tests with planktonic bacteria was substantially different from that of the adherent cells, colony counts were performed by plating representative planktonic and resuspended adherent isolates (see Table 2).

The MIC, MIC^{adh}, MBC, and MBC^{adh} measurements obtained are summarized in Table 1. The MICs of most antibiotics for 90% of the strains tested (MIC_{90}s) were comparable to the $MIC_{90}{}^{adh}$s. The MBC_{90} of the bactericidal antibiotic vancomycin (8 μg/ml) was fourfold lower than that of the bacteriostatic drugs tigecycline and minocycline (32 μg/ml) for freely growing cells. However, tigecycline and minocycline demonstrated more killing of adherent cells than did vancomycin and daptomycin ($MBC_{90}{}^{adh}$s, 8, 8, 32, and 32 μg/ml, respectively). The MBC^{adh}s of vancomycin and daptomycin were higher than the MBC for planktonic bacteria by 2 to 3 dilutions. Tigecycline, minocycline, and teicoplanin demonstrated better activity against the biofilm bacteria than did the other comparative agents. The only agent that was slightly more active against adherent *S. epidermidis* ($MBC_{90}{}^{adh}$, 4 μg/ml) was the mannopeptimycin antibiotic AC98-6446, a novel semisynthetic glycopeptide under development at Wyeth (P. Labthavikul, P. J. Petersen, T. Z. Wang, R. G. Dushin, and P. A. Bradford, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F355, 2002; R. G. Dushin and T. Z. Wang, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F352, 2002). There was no correlation between the amount of slime produced by and the antibiotic susceptibility

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TABLE 1. In vitro MICs and MBCs of tigecycline and comparative antibiotics against 68 planktonic and adherent *S. epidermidis* isolates

Antimicrobial agent	Planktonic bacteria (μg/ml)					Adherent bacteria (μg/ml)				
	MIC range	MIC_{50}	MIC_{90}	MBC range	MBC_{50}	MBC_{90}	MIC^{adh} range	MIC_{50}^{adh}	MIC_{90}^{adh}	MBC^{adh} range	MBC_{50}^{adh}	MBC_{90}^{adh}
Tigecycline	0.06–1	0.5	0.5	0.12–>32	4	32	≤0.015–1	0.25	0.5	1–8	4	8
Minocycline	0.06–1	0.5	1	0.25–>32	8	32	≤0.015–2	0.25	0.5	1–16	4	8
Vancomycin	1–8	2	4	1–16	4	8	≤0.015–4	2	4	2–>32	16	32
AC98–6446	0.06–2	0.25	0.5	0.06–4	0.5	1	0.12–2	1	2	1–8	2	4
Daptomycin	0.5–8	1	2	0.5–64	1	4	0.06–4	1	2	0.5–64	4	32
Teicoplanin	0.12–8	2	8	0.25–16	4	16	0.06–4	1	4	0.5–32	4	8
Gentamicin	≤0.015–>32	0.25	>32	0.03–>32	32	>32	≤0.015–32	4	>32	0.12–>32	32	>32
Tobramycin	0.06–>32	16	>32	0.06–>32	32	>32	0.06–>32	8	>32	0.12–>32	>32	>32
Ciprofloxacin	0.06–>32	0.25	32	0.12–>32	0.5	>32	0.06–>32	0.25	32	0.5–>32	2	>32
Oxacillin	≤0.06–>128	4	128	0.06–>128	8	>128	≤0.06–>128	2	128	0.12–>128	16	>128
Cefazolin	≤0.06–>128	2	32	≤0.06–>128	2	128	≤0.06–>128	1	16	0.5–>128	16	128
Rifampin	≤0.015–>8	≤0.015	>8	≤0.015–>8	0.03	>8	≤0.015–>8	≤0.015	>8	≤0.015–>8	0.03	>8
SXT[a]	0.12–>128	2	64	4–>128	>128	>128	0.12–128	1	64	1–>128	>128	>128

[a] SXT, trimethoprim-sulfamethoxazole.

TABLE 2. Inoculum concentration of *S. epidermidis*

Slime production group	CFU/ml	
	Planktonic bacteria	Adherent bacteria recovered from well
Strong producers		
S. epidermidis GC 5744	1.4×10^5	3.9×10^4
S. epidermidis PT 1031	2.0×10^5	5.6×10^5
Moderate producers		
S. epidermidis GC 5734	1.0×10^5	1.7×10^5
S. epidermidis GC 5746	3.2×10^5	2.0×10^5
Weak producers		
S. epidermidis GC 2794	6.0×10^5	4.8×10^5
S. epidermidis GC 5737	1.7×10^5	7.2×10^5
Nonproducers		
S. epidermidis PT 5368	2.0×10^5	8.0×10^3
S. epidermidis PT 7276	1.0×10^5	1.3×10^4

of strongly, moderately, and poorly slime-producing and non-slime-producing *S. epidermidis* bacteria. The colony counts from representative inocula used in the MIC tests with planktonic bacteria and bacteria recovered from resuspended adherent cells are shown in Table 2. For most of the strains, there was no significant difference between the inoculum used and the MICs determined with planktonic cells or adherent bacteria recovered from the wells. However, as expected, the colony counts of the two strains that did not produce slime were approximately 1 log lower than those of the adherent bacteria recovered from the wells.

Biofilm is an assemblage of bacteria and a polysaccharide matrix (slime) that allows the bacteria to adhere to smooth surfaces and medical devices. The embedded bacteria are less accessible to antibiotics and the human immune system defense mechanism. None of the currently used bactericidal-compound-based technologies is completely effective at preventing microbial colonization of medical catheters (4, 6, 8, 10). In this study, we demonstrated that the bactericidal activities of tigecycline and minocycline against adherent *S. epidermidis* in a biofilm model were better than their activities against freely growing cells in that the MBC_{90}^{adh}s were lower than the MBC_{90}s. Growth rate differences between adherent and planktonic cells have been indicated as a possible cause of susceptibility differences (2, 9, 10). This growth rate difference may have been a factor contributing to the better activity of tigecycline against adherent cells as well. The ability of tigecycline to inhibit the growth of adherent cells of *S. epidermidis* indicates that tigecycline is able to diffuse through the biofilm and act normally against its cellular target.

The findings of this in vitro study suggest that tigecycline might be considered for treatment of implant- or catheter-associated infections caused by slime-producing staphylococci. Although this model is lacking many of the components of a true in vivo biofilm, it is a good model with which to determine antibacterial activity against surface-associated bacteria. The relevance of any biofilm susceptibility testing to in vivo efficacy is still lacking. Therefore, further in vivo studies are warranted

to confirm the efficacy of tigecycline against staphylococcal biofilm.

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