

## Comparison of Tigecycline and Vancomycin for Treatment of Experimental Foreign-Body Infection Due to Methicillin-Resistant *Staphylococcus aureus*<sup>∇</sup>

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**Twice-daily 7-day regimens of tigecycline (7 mg/kg) and vancomycin (50 mg/kg) were compared in a rat tissue cage model of chronic foreign-body infection due to methicillin (meticillin)-resistant *Staphylococcus aureus* strain MRGR3. Subcutaneously administered tigecycline reached levels in tissue cage fluid that were nearly equivalent or slightly superior to the antibiotic MIC (0.5 µg/ml) for strain MRGR3. After 7 days, equivalent, significant reductions in bacterial counts were recorded for tigecycline-treated and vancomycin-treated rats, compared with those for untreated animals.**

Antimicrobial therapy for foreign-body infections due to *Staphylococcus aureus* is challenging (38), in particular for multidrug-resistant hospital-associated and community-acquired isolates of methicillin (meticillin)-resistant *S. aureus* (MRSA) (3, 12, 15, 16). Tigecycline is a novel injectable glycycline broad-spectrum antibiotic that demonstrates excellent in vitro and in vivo activity against MRSA and other multiresistant organisms (9, 11, 22, 28, 32) and can overcome both major tetracycline resistance mechanisms, namely ribosomal protection (10, 23) and efflux (4, 27). Tigecycline has shown good activity in various animal models of serious MRSA infections (21, 39, 40), as well as against biofilm-embedded bacteria (14, 26).

We previously used a rat tissue cage model of *S. aureus* chronic foreign-body infections for evaluating a number of antimicrobial agents, namely vancomycin (17), teicoplanin (31), imipenem (30), ceftobiprole (37), daptomycin (29, 35), and several fluoroquinolones (2, 17, 36). This study reports the activity of tigecycline compared to that of the reference anti-MRSA agent vancomycin in a tissue cage model of MRSA chronic foreign-body infection.

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MRSA strain MRGR3, whose properties were previously described (2, 5, 17, 29–31, 36, 37), was used for in vitro and in vivo studies. Strain MRGR3 is resistant to methicillin, gentamicin, erythromycin, tetracycline, and chloramphenicol (17).

MICs of freshly prepared (1, 13, 25) tigecycline (Wyeth Research, Collegeville, PA) or vancomycin (Vancocin; Teva Pharma AG, Switzerland) for MRSA strain MRGR3 or quality control *S. aureus* ATCC 29213 were determined by broth macrodilution in

cation-adjusted Mueller-Hinton broth (CAMHB), according to Clinical and Laboratory Standards Institute guidelines (7).

The animal protocol used for evaluating the in vivo activities of tigecycline and vancomycin was previously described in detail (17, 37) and approved by the Ethics Committee of the Faculty of Medicine, University of Geneva, and the Veterinary Office of the State of Geneva. Three weeks after subcutaneous implantation of four tissue cages per animal in anesthetized Wistar rats (37), tissue cage fluids were checked for sterility (17).

Pilot pharmacokinetic studies were performed using groups of noninfected rats to find an adequate dosing regimen of tigecycline for therapy of tissue cage infections as described previously (37). Tigecycline levels in cage fluids (and blood) were estimated by a microbiological assay (21), with a detection limit of 0.25 µg/ml. To account for protein binding, all plasma or tissue cage fluid samples were diluted with 1 volume of phosphate-buffered saline and assayed in duplicate, with reference to duplicate standard concentrations (0.25 to 8 µg/ml) of tigecycline, in phosphate-buffered saline supplemented with 50% plasma or pooled tissue cage fluids, respectively.

Each tissue cage was chronically infected by inoculating  $5 \times 10^5$  CFU of log-phase MRGR3 (37). Two weeks later, all rats whose cage fluids contained  $\geq 10^5$  CFU/ml received twice-daily doses (by the subcutaneous route for 7 days) of tigecycline (7 mg/kg), vancomycin (50 mg/kg), or no antibiotic (control group). Differences in CFU counts of cage fluid quantitative cultures, performed at day 1 (before treatment) and day 8 (12 h after the last injection of either tigecycline or vancomycin), were expressed as the change in number of  $\log_{10}$  CFU/ml (37) and evaluated by one-way analysis of variance and post-analysis of variance pairwise comparisons between individual groups via the Tukey HSD test (<http://faculty.vassar.edu/lowry/VassarStats.html>), using *P* values of <0.05 with two-tailed significance levels.

Tigecycline resistance was screened by plating 10-fold-diluted cage fluids (100 µl) onto MH agar supplemented with 2 µg/ml tigecycline. No single colony grew on tigecycline-supple-

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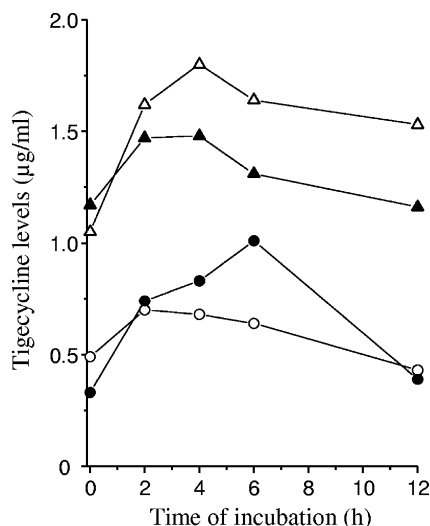


FIG. 1. Pharmacokinetic levels of tigecycline in tissue cage fluids of rats on day 4 (open symbols) or day 7 (closed symbols) of therapy every 12 h with 7 mg/kg (○) or 14 mg/kg (△) of tigecycline. Each value is the mean result of six determinations.

mented plates inoculated with  $10^8$  CFU of in vitro-grown cultures of strain MRGR3.

The MIC of tigecycline in CAMHB for MRSA strain MRGR3 was 0.5  $\mu\text{g/ml}$ , namely at the upper limit of susceptibility breakpoints (7), and was unaffected by supplementation of CAMHB with 50% tissue cage fluid (data not shown). Since tigecycline did not produce a 3- $\log_{10}$  reduction in the number of MRGR3 CFU/ml, it was not considered bactericidal. Nevertheless, supra-MIC levels (1, 2, and 4  $\mu\text{g/ml}$ ) of tigecycline produced a 2- to 3- $\log_{10}$  decrease in the number of MRGR3 CFU/ml at 24 h. The vancomycin MIC and minimal bactericidal concentration for strain MRGR3 were 1 and 2  $\mu\text{g/ml}$ , respectively (17).

Average tigecycline levels, scored for tissue cage fluids ( $n = 6$ ) from 0 to 12 h after subcutaneous administration, remained quite constant over time, showing  $\leq 3$ -fold variations between results at different time points and moderate animal-to-animal differences (Fig. 1). A 7-mg/kg twice-daily regimen yielded cage fluid levels of 0.39 to 0.70  $\mu\text{g/ml}$  tigecycline at day 4 and 0.33 to 1.01  $\mu\text{g/ml}$  at day 7, such results thus being nearly equivalent or slightly superior to the antibiotic MIC for MRGR3. Tigecycline plasma levels at 2 h on day 4 were  $1.87 \pm 0.66$   $\mu\text{g/ml}$ , in agreement with other reports (8, 21). A 14-mg/kg twice-daily regimen led to plasma and tissue cage fluid tigecycline levels ca. twofold higher than the 7-mg/kg regimen (Fig. 1). Average peak and trough cage fluid levels of vancomycin were previously determined (17) as 12 and 2  $\mu\text{g/ml}$  at 4 and 12 h, respectively.

At day 1, mean bacterial counts for MRGR3-infected cages were not significantly different ( $P = 0.65$ ) in controls ( $6.85 \pm 0.19 \log_{10}$  CFU/ml;  $n = 28$ ), tigecycline-treated rats ( $6.92 \pm 0.13 \log_{10}$  CFU/ml;  $n = 29$ ), or vancomycin-treated rats ( $6.70 \pm 0.18 \log_{10}$  CFU/ml;  $n = 27$ ). At day 8, significant ( $P < 0.01$  versus controls) reductions were recorded in bacterial counts in cage fluids of both tigecycline-treated ( $-0.62 \pm 0.17 \log_{10}$  CFU/ml;  $n = 29$ ) and vancomycin-treated ( $-0.76 \pm 0.18 \log_{10}$

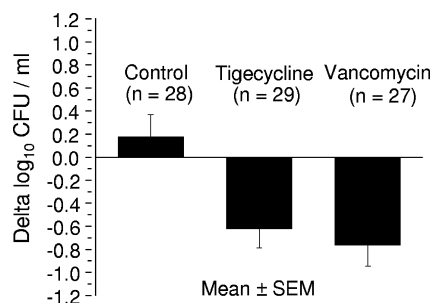


FIG. 2. Decrease in viable counts of MRSA MRGR3 in tissue cage fluids of rats treated for 7 days with tigecycline or vancomycin.

CFU/ml;  $n = 27$ ) rats, whereas the bacterial counts for controls slightly increased ( $+0.18 \pm 0.19 \log_{10}$  CFU/ml;  $n = 28$ ) (Fig. 2). The reductions in CFU counts for vancomycin-treated and tigecycline-treated rats were not significantly different. Finally, no MRGR3 isolate showing increased tigecycline MIC was observed in any posttherapy cage fluid sample ( $n = 29$ ). The lack of emergence of MRGR3 derives with diminished susceptibility to tigecycline is consistent with the difficulty in selecting laboratory-derived, tigecycline-resistant mutants of *S. aureus* (18), and it contrasts with the emergence of resistant subpopulations during low-dose daptomycin therapy of *S. aureus*-infected tissue cages (35).

Several studies performed with the rat tissue cage model demonstrated the low initial in vivo response of foreign-body-associated chronic MRSA infections (2, 5, 6, 17, 20, 29–31, 35–37). A much greater reduction of viable MRSA counts in cage fluids requires longer periods of antibiotic therapy (5), as found in clinical situations with foreign-body infections (38). Major pharmacokinetic properties of tigecycline, observed in human and animal studies, are very low plasma levels, long half-lives, and high volumes of distribution indicating extensive tigecycline distribution into the tissues (8, 11, 19, 28, 32, 40). In line with previous observations that showed a requirement for active, preferentially bactericidal, antibiotic levels for obtaining significant reductions of CFU counts in MRSA-infected cage fluids (29, 37), we selected for therapy a twice-daily 7-mg/kg regimen yielding cage fluid tigecycline levels above the MIC for strain MRGR3 for  $>50\%$  of the dosing interval (32, 33), while minimizing the occurrence of side effects previously observed with higher-dose regimens (39). Our regimen is similar to those required for activity in other animal models of hard-to-treat *S. aureus* infections, such as endocarditis or osteomyelitis (21, 39), although its relevance to human therapy is not fully defined (32). In addition, the incomplete in vitro killing activity of tigecycline, namely a  $<3$ - $\log_{10}$  reduction in number of MRGR3 CFU at 24 h, prevents a pharmacodynamic analysis of tigecycline in vivo activity more detailed than those of previously evaluated bactericidal antibiotics in MRSA-infected cages (29, 37). We can also speculate that other properties of tigecycline, namely its in vivo activity against intracellular, slowly growing, or biofilm-forming bacteria, might significantly contribute to tigecycline activity in MRSA-infected cages (34). Indeed, high intracellular levels of tigecycline were shown to accumulate in human polymorphonuclear neutrophils and prevent growth of phagocytized bac-

teria (24). Further studies are needed to elucidate the mechanisms of tigecycline activity against hard-to-treat MRSA infections.

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