Tigecycline Exhibits Inhibitory Activity against Clostridium difficile in the Colon of Mice and Does Not Promote Growth or Toxin Production

Robin L. P. Jump,1,2,3 Yuejin Li,2 Michael J. Pultz,2 Georgios Kypriotakis,1,4 and Curtis J. Donskey1,2,3,*

Geniatric Research, Education and Clinical Center,1 and Research Service,2 Louis Stokes Cleveland Veterans Affairs Medical Center and Division of Infectious Disease,3 Department of Medicine,4 Case Western Reserve University School of Medicine, Cleveland, Ohio

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Tigecycline is a broad-spectrum glycylcycline antibiotic with potent in vitro activity against Clostridium difficile. We used a mouse model to test the hypothesis that tigecycline has a low propensity to promote colonization and toxin production by C. difficile due to inhibitory activity in the colon. Mice (5 to 8 per group) received subcutaneous injections of tigecycline (low and high doses) alone or in combination with clindamycin for 6 days. Growth of and toxin production by 3 strains of C. difficile (tigecycline MICs ≤ 0.012 µg/ml) were measured on cecal contents collected 6 h or 3 days after the final antibiotic dose. Antibiotic concentrations were measured using a bioassay, and concentrations of total anaerobes and Bacteroides spp. were measured. The effects of tigecycline on rendering mice susceptible to colonization with and reducing the burden of C. difficile were also examined. In comparison to saline controls, clindamycin promoted the growth of C. difficile (P < 0.001) in cecal contents, whereas tigecycline did not. Tigecycline did not suppress total anaerobes or Bacteroides spp. in comparison to saline controls. Concurrent administration of tigecycline prevented clindamycin-induced promotion of C. difficile in cecal contents collected 6 h or 3 days (high dose only) after the final antibiotic dose. Tigecycline did not promote the establishment of colonization in mice, yet it did not reduce concentrations of C. difficile in animals with established colonization. In summary, tigecycline did not promote the growth of or toxin production by C. difficile, probably due to inhibitory activity against C. difficile and relative sparing of indigenous anaerobic microflora.

Antimicrobial therapy plays a central role in the pathogenesis of Clostridium difficile infection (CDI). The presumed mechanism by which antibiotics induce CDI is through disruption of the indigenous microflora of the colon, thereby allowing C. difficile to grow to high concentrations with production of toxin (13). Although nearly all classes of antibiotics have been associated with CDI, clindamycin, broad-spectrum cephalosporins, and penicillins have traditionally been considered the agents that pose the greatest risk (13). With the emergence of the North American pulsed-field gel electrophoresis type 1 (NAP1) epidemic strain of C. difficile that exhibits increased resistance to fluoroquinolone antibiotics, fluoroquinolones have also been associated with CDI in multiple studies (1, 9, 13). However, there remains some uncertainty regarding the relative importance of fluoroquinolones as a risk factor for CDI because these agents cause only minor disruption of intestinal anaerobes (1, 13). Some recent studies suggest that antibiotics with inhibitory activity against C. difficile may be less likely to promote CDI (1–3, 7, 13–15, 17–19). For example, piperacillin-tazobactam, a beta-lactam/beta-lactamase inhibitor combination, not only disrupts the indigenous intestinal microflora but also has potent activity against C. difficile and has been infrequently associated with CDI in clinical studies (15, 19). In mice, piperacillin-tazobactam inhibited growth of C. difficile in the colon during treatment but facilitated growth and toxin production when exposure occurred after treatment during the period of recovery of the indigenous microflora (14).

Tigecycline, a broad-spectrum glycylcycline antibiotic, also has potent activity against C. difficile and is excreted in significant concentrations in bile (median fecal concentration in human volunteers, 5.6 mg/kg of body weight on day 8 of administration) (12). In a chemostat model of the human intestinal microflora, tigecycline markedly decreased concentrations of bacteroides and bifidobacteria but did not induce proliferation or toxin production by C. difficile (3). Wilcox (17) has noted that tigecycline has infrequently been associated with CDI in clinical studies. Moreover, Herpers et al. recently reported successful use of intravenous tigecycline as salvage therapy for 4 patients with refractory CDI (7). These data suggest that tigecycline may achieve sufficient concentrations in the intestinal tract to inhibit the growth of C. difficile in this study, we used a mouse model to examine the effect of tigecycline on the growth of and toxin production by C. difficile. We hypothesized that the effect of tigecycline on C. difficile in the colon is similar to the effect of piperacillin-tazobactam: inhibition of growth and toxin production during tigecycline treatment but promotion of growth when exposure occurs after treatment during the period of recovery of the indigenous microflora.

MATERIALS AND METHODS

C. difficile strains. Three strains of C. difficile were studied. ATCC 43593 is a nontoxigenic strain from the American Type Culture Collection (ATCC). The other strains were cultured from patients with CDI in Cleveland, OH. VA 17 is an epidemic North American pulsed-field gel electrophoresis type 1 (NAP1) strain. VA 11 is a restriction endonuclease analysis (REA) J-type strain. For all three strains, the MICs of tigecycline, piperacillin-tazobactam, and clindamycin

* Corresponding author. Mailing address: Infectious Diseases Section (1110 W), Louis Stokes Cleveland Veterans Affairs Medical Center, 10701 East Blvd., Cleveland, OH 44106. Phone: (216) 791-3800, ext. 6153. Fax: (216) 231-3482. E-mail: curtisd123@yahoo.com.

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FIG. 1. Mouse model of in vitro colonization resistance to C. difficile. Mice (n = 5 per group) received daily subcutaneous antibiotics for 4 days. Six hours or 3 days after the final antibiotic dose, cecal contents were collected, and aliquots were inoculated with 10^4 CFU/ml of C. difficile. Samples were incubated anaerobically for 24 h and then plated onto selective media to quantify C. difficile (lower limit of detection, 2.5 log_{10}CFU/ml). Data for three strains of C. difficile were pooled. Error bars represent standard errors of the means. Pip = piperacillin. Detection of C. difficile toxin by C. difficile Tox A/B II (Wampole Laboratories) is indicated below the x axis. *, P < 0.001 versus saline.

FIG. 2. Effect of antibiotic treatment on concentrations of total anaerobes and Bacteroides spp. Mice (n = 6) received daily subcutaneous antibiotic treatment for 4 days. Six hours after the final dose, cecal contents were collected and plated anaerobically onto prereduced bruccella agar to quantify total anaerobes (A) and onto Bacteroides bile-esculin agar to quantify Bacteroides spp. (B). The lower limit of detection was 4 logs. Error bars represent standard errors of the means. Tige = tigecycline; Pip-Tazo = piperacillin-tazobactam; Clinda = clindamycin. *, P < 0.001 versus saline.
levels of tigecycline in cecal contents collected 6 h after the final dose on day 4 were 2.8 ± 0.4 and 9.0 ± 3.4 μg/ml (± standard errors of the means [SEM]) at the human-equivalent (mg/kg) and the 12-times-higher doses, respectively. Tigecycline was not detected in the cecal contents of mice receiving either dose when collected 3 days after the final dose.  

**Mouse model of in vivo C. difficile colonization.** Figure 3 shows the effect of antibiotic treatment on the establishment of colonization with the nontoxigenic *C. difficile* strain ATCC 43593. In comparison to saline controls, clindamycin-treated mice developed high concentrations of *C. difficile* in stool (*P* < 0.001), whereas mice receiving either the human-equivalent dose or the 12-times-higher dose of tigecycline did not.  

Figure 4 shows the assessment of whether antibiotic treatment results in suppression of *C. difficile* levels after colonization has been established. Subcutaneous injection of piperacillin-tazobactam resulted in a significant decrease in *C. difficile* concentrations in stool during treatment (P of <0.05 on days 2 and 5). Oral vancomycin also caused a decrease in stool concentrations of *C. difficile* that was significant on day 2 (*P* < 0.05) and approached significance on day 5 (*P* = 0.058). After treatment discontinuation, *C. difficile* levels in the groups of mice rapidly rebounded to pretreatment levels. Tigecycline treatment did not reduce *C. difficile* concentrations in stool in comparison to saline controls.

**DISCUSSION**

We found that tigecycline did not promote in vitro growth of or toxin production by *C. difficile* in cecal contents collected from mice during or 3 days after completion of treatment. As in previous studies, piperacillin-tazobactam inhibited growth of *C. difficile* during treatment but promoted overgrowth in cecal contents collected 3 days after completion of treatment (14). Clindamycin promoted overgrowth of clindamycin-resistant *C. difficile* in cecal contents during and after treatment. In previous studies, clindamycin inhibited the growth of clindamycin-susceptible strains during, but not after, treatment (1). Piperacillin-tazobactam and clindamycin treatment reduced concentrations of total anaerobes and *Bacteroides* spp. in cecal contents, whereas tigecycline treatment did not. For each of the antibiotics studied, the levels detected in mouse stool or cecal contents are comparable to concentrations detected in stool samples of healthy human volunteers or patients receiving the same antibiotics (6, 8, 11, 18). Our findings suggest that tigecycline might have a relatively low propensity to promote CDI due to inhibitory activity against *C. difficile* and relative sparing of indigenous anaerobic microflora.

Our results are consistent with some findings of previous studies in human volunteers and using a chemostat model of human intestinal microflora (3, 12). As in mice, tigecycline did not inhibit *Bacteroides* spp. in healthy human adults (12). Bifidobacteria were significantly reduced in human volunteers (12), but levels of bifidobacteria were not measured in the current study. In the chemostat model, instillation of tigecycline resulted in a 3- to 4-log decrease in total anaerobes and marked suppression of bacteroides, which failed to recover during the 14-day postantibiotic period (3). Despite the reduction in anaerobic microflora in the chemostat model, instillation of tigecycline was not associated with germination or outgrowth of *C. difficile* spores, suggesting that growth was inhibited (3).

Tigecycline treatment did not promote *C. difficile* colonization in mice, yet it did not reduce *C. difficile* levels in animals with preestablished colonization. In contrast, piperacillin-tazobactam and oral vancomycin suppressed levels of *C. difficile* in mice with preestablished colonization. The concentration of the antibiotics in the intestinal tract may explain the
differing effects of the 3 agents. Compared to piperacillin-
tazobactam and vancomycin levels determined in previous
mouse model studies (piperacillin-tazobactam, 31.2 ± 9.7 µg
and 2.0 ± 0.8 µg/ml of cecal contents at 6 h and 3 days after
discontinuation of treatment, respectively; vancomycin, >500
µg/ml of cecal contents at both time points) (13; our unpub-
lished data), the peak fecal concentration of tigecycline was
much lower (2.8 ± 0.4 µg/gm), and no drug was detectable 3
days after discontinuation of treatment. Although tigecycline
achieves peak concentrations in stool several times above the
typical MICs of C. difficile isolates, factors such as rapid clear-
ance from the intestinal tract, an inoculum effect (i.e., intesti-
nal concentrations above the 10^5 inoculum used for MIC test-
ing), or binding to fecal matter could potentially reduce its in
vivo effects in the intestinal tract (13).

In conclusion, tigecycline did not promote growth of or toxin
production by C. difficile, probably due to inhibitory activity
against C. difficile and relative sparing of indigenous anaerobic
microflora.

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