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Tigecycline Displays *In Vivo* Bactericidal Activity against Extended-Spectrum-β-Lactamase-Producing *Enterobacteriaceae* after 72-Hour Exposure Period

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Progressively enhanced activity of a humanized tigecycline (TGC) regimen was noted over 3 days against an extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* isolate and an ESBL-producing *Klebsiella pneumoniae* isolate. Bacterial density reduction approximated 3 $\log_{10}$ approaching bactericidal activity at 72 h. This level of activity has not been previously noted for compounds such as tetracyclines, normally considered bacteriostatic antimicrobials. Extended regimen studies *in vivo* may aid in better delineation of antimicrobial effects, producing improved correlation with clinical outcomes.

Historically, *in vitro* and *in vivo* pharmacodynamic (PD) assessments have been conducted over 24 h. While these studies have been noted to correlate with clinical outcomes for rapidly bactericidal agents (i.e., fluoroquinolones or aminoglycosides), these PD endpoints appear more poorly correlated for agents such as the tetracyclines and related derivatives which have slower killing profiles *in vitro*. The tetracyclines and the class-related extended-spectrum agents, such as tigecycline (TGC), have demonstrated bacteriostatic activity in *in vitro* studies against a variety of bacterial strains (1). In a recent study using the endpoint of reduction in numbers of CFU after 24 h of TGC exposure against several *Escherichia coli* and *Klebsiella pneumonia*e isolates, we noted antibacterial activity in both immunocompromised and immunocompetent mice (2). The majority of doses were bacteriostatic at best. The maximum reduction in numbers of CFU was 2 $\log_{10}$ over this 24-h exposure period; however, these effects were noted only when exposures were well above that typically seen in humans. In addition, it was also noted that the *in vivo* exposures required to produce substantial reductions in numbers of CFU in the immunocompromised murine model were well in excess of that recognized to produce good clinical and microbiologic outcomes in patients (2). While the immunocompromised model appeared to have exposures discordant to that observed in humans, the immunocompetent model required exposures similar to that observed in patients. Therefore, in the current study, we sought to determine the magnitude of bacterial kill over an extended treatment period of 72 h using an exposure in immunocompetent animals that would mimic the regimen of a 100-mg loading dose with subsequent 50-mg doses every 12 h (q12h) of TGC in humans.

Two extended-spectrum-β-lactamase (ESBL)-producing clinical strains, one *E. coli* (*E. coli* strain 363; TGC MIC, 0.125 μg/ml) and one *K. pneumoniae* (*K. pneumoniae* strain 404; TGC MIC, 0.25 μg/ml), provided by Tetraphase Pharmaceuticals, Inc. (Watertown, MA), were tested in an immunocompetent thigh infection model. ICR (CD-1) mice weighing approximately 25 g were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). These studies were approved by and followed the guidelines of the Institutional Animal Care and Use Committee at our facility. Bacterial colonies of a fresh subculture of each isolate were suspended in sterile 0.9% sodium chloride to produce a suspension of approximately 10^8 CFU/ml. Final inoculum concentrations were confirmed by plating serial dilutions of inocula on Trypticase soy agar with 5% sheep blood (BD Biosciences, Sparks, MD) and incubating plates at 35°C overnight. Both hind legs of each mouse were injected with 0.1 ml of the above-described bacterial suspension (approximately 10^7 CFU) of one of the test isolates. The first TGC dose was administered at two hours postinoculation. Each bacterial isolate was tested once over a 72-h period.

Tigecycline powder was obtained from Pfizer, Inc. (Groton, CT), and solubilized in normal saline immediately prior to dosing. The dosing regimen that would produce a drug exposure *in vivo* that is similar to that in humans, quantified by the area under the concentration-time curve from 0 to 24 h ($AUC0\text{-}_{24}$) in blood, was replicated (3, 4). As such, a free (unbound) TGC $AUC_{0\text{-}24}$ of 1.13 (or a total—bound and unbound—TGC $AUC_{0\text{-}24}$ of 5.39 with 79% protein binding), observed in humans following the regimen of a 100-mg loading dose with subsequent 50-mg doses q12h (4, 5), was simulated over each of the three 24-h intervals in the mice. The *in vivo* regimen utilized in this current study was 12.5 mg/kg q24h, administered via subcutaneous (s.c.) injection, which was previously determined to produce a drug exposure similar to that in humans (2, 6). This regimen was administered to mice daily for a 3-day period (72 h) or until tissue harvest. Prior to dose administration, one group of mice ($n = 3$) for each bacterial isolate was sacrificed and thigh tissues were harvested to assess the initial CFU level. Thighs from one group each ($n = 3$) of control (vehicle-dosed) and TGC-treated mice infected with *E. coli* 363 or *K. pneumoniae* 404 were harvested and processed for quantitative culture after 24, 48, and 72 h of treatment. For tissue harvest, both rear thighs were removed from each mouse and individually homogenized in 5 ml sterile normal saline. Dilutions of the thigh homogenates were plated on Trypticase soy agar with 5% sheep blood agar plates (BD Biosciences) and incubated at approximately 37°C

Received 4 September 2012 **Returned for modification** 25 September 2012
Accepted 28 October 2012
Published ahead of print 31 October 2012
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doi:10.1128/AAC.01824-12

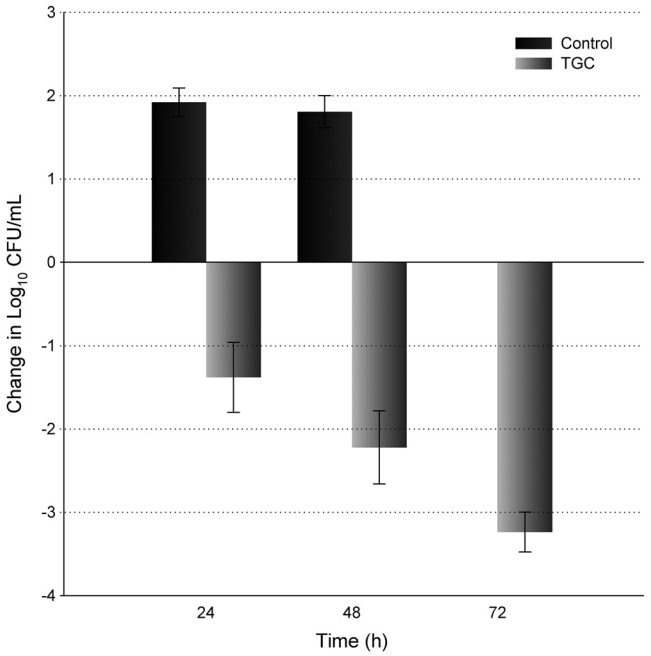

FIG 1 Reduction in bacterial density of *E. coli* 363 over 72 h after administration of TGC in immunocompetent mice. Black bars, change in bacterial burden in control groups; gray bars, change in bacterial burden in TGC treatment groups relative to untreated controls 2 h postchallenge. Error bars indicate standard deviations. At 48 h, all remaining control animals perished, so the two groups of control animals (48 h and 72 h) were grouped together ($n = 6$).

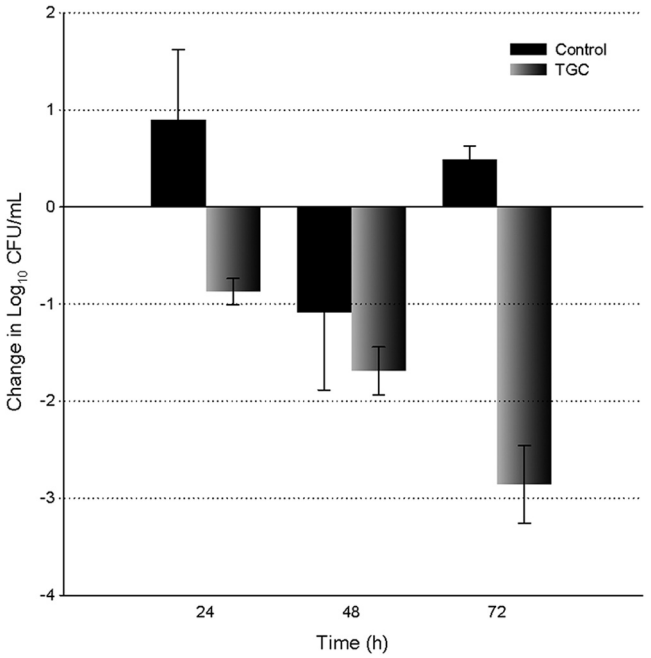

FIG 2 Reduction in bacterial density of *K. pneumoniae* 404 over 72 h after administration of TGC in immunocompetent mice. Black bars, change in bacterial burden in the control groups; gray bars, change in bacterial burden in TGC treatment groups relative to untreated controls 2 h postchallenge. Error bars indicate standard deviations.

in ambient air overnight. Efficacy was calculated as the change in bacterial density ($\Delta\log_{10}$ numbers of CFU) obtained in the TGC-treated mice after 24, 48, and 72 h relative to that of the 0-h untreated controls for *E. coli* 363 and *K. pneumoniae* 404.

Twenty-four hours after inoculation, the bacterial density of *E. coli* 363 increased 1.9 $\log_{10}$ in the untreated control animals. After the initial day of TGC exposure, a reduction of 1.4 $\log_{10}$ in bacterial density was quantified. Unfortunately, all of the control animals randomized to the 48- and 72-h control groups perished by 48 h. As a result, CFU data from these animals were combined into the 48-h control group as noted in Fig. 1. All TGC-treated animals survived until the designated tissue harvest interval; thus, the remaining TGC treatment groups were evaluated at 48 h and 72 h. On the 2nd (48 h) and 3rd (72 h) days of treatment, the TGC regimen produced a cumulative $\log_{10}$ reduction in numbers of CFU of 2.2 and 3.2, respectively (Fig. 1).

The numbers of CFU of *K. pneumoniae* 404 increased by 0.9 $\log_{10}$ after 24 h in the untreated control animals. The growth of this strain was variable at 48 h (e.g., in the untreated control group, CFU range of +0.1 to −1.6 $\log_{10}$) with a net negative average number of CFU; however, sustained growth was noted at 72 h in those animals randomized to the later time point (Fig. 2). An increasing effect of the TGC treatment was observed over the treatment period, as noted by the $\log_{10}$ reduction in numbers of CFU of 0.9, 1.7, and 2.9 at 24 h, 48 h, and 72 h, respectively, after initiation of therapy (Fig. 2). The combined $\log_{10}$ reduction in numbers of CFU for both *E. coli* 363 and *K. pneumoniae* 404 averaged 1.1, 2.0, and 3.0 at 24 h, 48 h, and 72 h, respectively.

The human simulated exposures of TGC utilized in this current investigation resulted in enhanced antibacterial activity against both isolates *in vivo* with each subsequent day of therapy. Exposures of TGC equivalent to a 100-mg loading dose and subsequent 50-mg doses q12h in humans exhibited antibacterial activity of about 1 $\log_{10}$ bacterial kill at 24 h in this infection model, as might have been anticipated (2, 6); however, this efficacy was improved on each subsequent day of dosing. After 3 days of treatment, these cumulative TGC exposures had a pronounced effect on the order of a 3-$\log_{10}$ reduction in numbers of CFU compared to the numbers of CFU in control animals 2 h postchallenge, displaying bactericidal activity.

Based on *in vitro* time-kill studies as well as *in vivo* assessments, the ribosomally mediated antimicrobials, such as macrolides, tetracyclines, and oxazolidinones, have been considered bacteriostatic. To this point, we also noted *in vivo* bacteriostatic activity after humanized doses of clarithromycin versus *Streptococcus pneumoniae* after 24 h (7). While the 24-h data derived in the current study point to a similar bacteriostatic endpoint, continued TGC exposures produced bactericidal activity after 72 h. Furthermore, in a recent study, we noted that 24 h of humanized dose regimens of tedizolid and linezolid produced approximately 1- to 2-$\log_{10}$ reductions in numbers of CFU in an *in vivo* immunocompetent lung infection model against methicillin-resistant *Staphylococcus aureus* (8). When we administered these humanized regimens of tedizolid and linezolid over a 72-h period *in vivo*, both compounds produced bactericidal effects against methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* in an immunocompetent thigh infection model (7).

These studies provide insight into the *in vivo* potency and the magnitude of antimicrobial effects of tigecycline over an extended treatment period of 72 h in an immunocompetent host against two ESBL-producing *Enterobacteriaceae*. Notably, we observed an

enhanced cumulative reduction in bacterial density over 72 h. While this compound is not typically regarded as bactericidal *in vitro* (i.e., producing a 3-$\log_{10}$ reduction in numbers of CFU), these *in vivo* results suggest that a bactericidal endpoint can be achieved over the 3-day treatment regimen with humanized doses used in this current study. The *in vivo* killing profile of tigecycline observed here indicates that assessments of the PD profile beyond 24 h may provide a more predictive value when considering the potential clinical efficacy of this compound or other structurally related investigational compounds in the management of infections in humans.

ACKNOWLEDGMENTS

This work was funded by a grant from Tetraphase Pharmaceuticals, Inc.

David P. Nicolau has acted as a consultant for and received research grants from Tetraphase Pharmaceuticals and Pfizer. Pamela R. Tessier has no conflict of interest to disclose.

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