Pharmacodynamics of TD-1792, a Novel Glycopeptide-Cephalosporin Heterodimer Antibiotic Used against Gram-Positive Bacteria, in a Neutropenic Murine Thigh Model

Sharath S. Hegde,[a] Olanrewaju O. Okusanya,[b] Robert Skinner,[a] Jeng-Pyng Shaw,[a*] Glenmar Obedencio,[a] Paul G. Ambrose,[b] Johanne Blais,[a] and Sujata M. Bhavnani[b]

Theravance, Inc., South San Francisco, California, USA,[a] and Institute for Clinical Pharmacodynamics, Latham, New York, USA[b]

TD-1792 is a novel glycopeptide-cephalosporin heterodimer investigational antibiotic that displays potent bactericidal effects against clinically relevant Gram-positive organisms *in vitro*. The present studies evaluated the *in vivo* pharmacokinetics (PK) and pharmacodynamics (PD) of TD-1792 in the neutropenic murine thigh infection animal model. TD-1792, dosed subcutaneously (SC), produced dose-dependent reduction in the thigh bacterial burden of several organisms, including methicillin-susceptible and -resistant strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* (MSSA, MRSA, MSSE, MRSE, respectively), penicillin-susceptible strains of *Streptococcus pneumoniae* (PSSP), *Streptococcus pyogenes*, and vancomycin-intermediate-susceptible *Staphylococcus aureus* (VISA). In single-dose efficacy studies, the 1-$\log_{10}$ CFU kill effective dose ($ED_{1\text{-log kill}}$) estimates for TD-1792 ranged from 0.049 to 2.55 mg/kg of body weight administered SC, and the bacterial burden was reduced by up to 3 $\log_{10}$ CFU/g from pretreatment values. Against *S. aureus* ATCC 33591 (MRSA), the total 24-h $\log_{10}$ stasis dose (ED_{stasis}) and $ED_{1\text{-logkill}}$ doses for TD-1792 were 0.53 and 1.11 mg/kg/24 h, respectively, compared to 23.4 and 54.6 mg/kg/24 h for vancomycin, indicating that TD-1762 is 44- to 49-fold more potent than vancomycin. PK-PD analysis of data from single-dose and dose-fractionation studies for MRSA (ATCC 33591) demonstrated that the total-drug 24-h area under the concentration-time curve-to-MIC ratio (AUC/MIC ratio) was the best predictor of efficacy ($r^2 = 0.826$) compared to total-drug maximum plasma concentration of drug-to-MIC ratio (Cmax/MIC ratio; $r^2 = 0.715$) and percent time that the total-drug plasma drug concentration remains above the MIC (%Time>MIC; $r^2 = 0.749$). The magnitudes of the total-drug AUC/MIC ratios associated with net bacterial stasis, a 1-$\log_{10}$ CFU reduction from baseline and near maximal effect, were 21.1, 37.2, and 51.8, respectively. PK-PD targets based on such data represent useful inputs for analyses to support dose selection decisions for clinical studies of patients.

Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), are a major cause of serious nosocomial infections worldwide (18). The glycopeptide vancomycin still serves as the cornerstone in the treatment of drug-resistant Gram-positive infections, but its microbiological limitations are a growing concern (9). The introduction of newer agents, including linezolid, daptomycin, tigecycline, telavancin, and ceftaroline, has offered additional therapeutic options to the clinician (16). Nevertheless, given the recognized therapeutic limitations of existing drugs and the threat of emerging resistance, the need for new antibiotics that operate through novel mechanisms remains high.

TD-1792 (Fig. 1) is a bactericidal multivalent glycopeptide-cephalosporin antibiotic that simultaneously targets the D-Ala-D-Ala-containing peptidoglycan precursors and the active site of penicillin-binding protein, resulting in potent activity against Gram-positive bacteria, including multiresistant strains (10, 13, 14, 15). The objective of the present studies was to characterize the pharmacodynamics of TD-1792 against a collection of Gram-positive pathogens in the neutropenic murine thigh model of soft tissue infection. In addition, pharmacokinetic-pharmacodynamic (PK-PD) studies were also conducted to elucidate the PK-PD index associated with TD-1792 efficacy and the magnitude of such an index required to achieve various levels of reduction in bacterial burden.

MATERIALS AND METHODS

Antimicrobial agents. TD-1792 was synthesized at Theravance, Inc., South San Francisco, CA. Vancomycin was obtained from Sigma-Aldrich (St. Louis, MO). Both drugs were dissolved in 5% dextrose in water for dosing.

Bacterial strains. Eight strains were evaluated in this study. These strains tested included an MRSA strain (ATCC 33591), a methicillin-susceptible *S. aureus* (MSSA) strain (ATCC 13709), a methicillin-resistant *Staphylococcus epidermidis* (MRSE) strain (MED 820), a methicillin-susceptible *S. epidermidis* (MSSE) strain (SU03), two penicillin-susceptible *Streptococcus pneumoniae* (PSSP) strains (MED 35 and MED 1119), a *Streptococcus pyogenes* strain (MED 2040), and a vancomycin-intermediate-susceptible *S. aureus* (VISA) strain (HIP5836). The MRSA and MSSA strains (ATCC 33591 and 13709, respectively) were obtained from the American Type Culture Collection (Manassas, VA). All other strains were obtained from U.S. hospitals or academic institutions.

***In vitro* susceptibility testing.** MICs were determined using the CLSI broth microdilution method (6). Studies to investigate the effect of serum on *in vitro* activity were carried out by performing susceptibility testing against MRSA (ATCC 33591) in the presence of 50% (vol/vol) mouse serum.

Protein binding determination. The *in vitro* binding of TD-1792 to mouse and human plasma proteins for concentrations ranging from 0.1 to 10 μg/ml was evaluated by ultrafiltration using a Centrifree micropartition device. Samples (1 ml) of spiked plasma containing ^{14}C-TD-1792
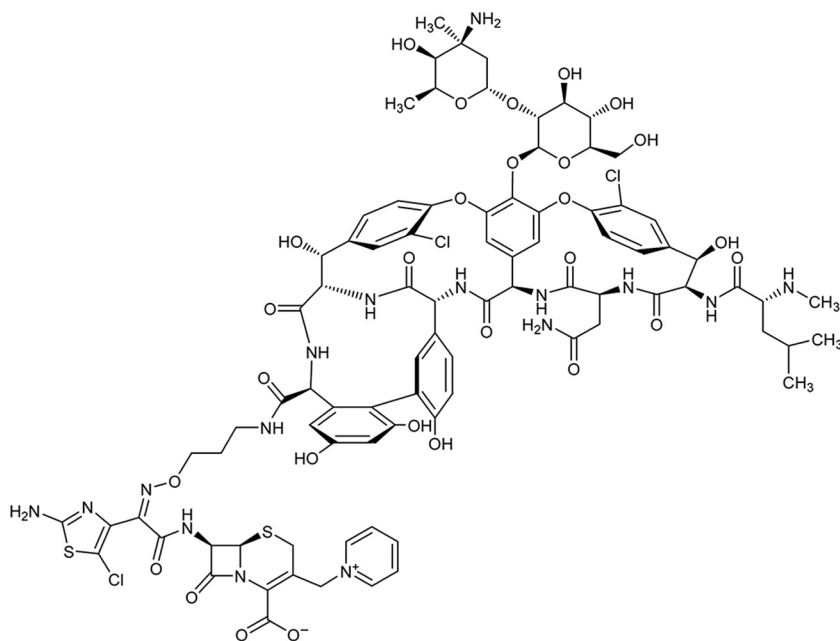
FIG 1 Chemical structure of TD-1792.

and unlabeled TD-1792 were loaded into the Centrifree micropartition devices with a 30,000-molecular-weight-cutoff regenerated cellulose membrane and then centrifuged at 1,000 × *g* for 20 min at 37°C. The fortified plasma and the resulting protein-free ultrafiltrate were transferred into a scintillation cocktail and counted with a beta counter.

Efficacy studies in a murine neutropenic thigh infection model. All animal studies were approved by the Institutional Animal Care and Use Committee at Theravance, Inc., and conducted in the Association for Assessment and Accreditation of Laboratory Animal Care-approved animal facility at Theravance, Inc.

The experimental methodology and design were similar to that described previously (12). Animals (female NSA mice, 18 to 30 g) were acquired from Charles Rivers Laboratories (Gilroy, CA). Mice were housed 5 per cage and allowed at least 3 days after their arrival date to acclimate before the study was initiated. Throughout the study, animals had access to food and water *ad libitum*. Neutropenia (approximately 100 cells/mm³) was induced by administering 200 mg of cyclophosphamide per kg of body weight intraperitoneally 4 and 2 days prior to inoculation of bacteria.

Animals were lightly anesthetized with isoflurane (2.5% for induction followed by 1% for maintenance), and 50 μl of the bacterial inoculum was injected into the posterior thigh. The bacterial inoculum administered ranged from 10^6 to 10^7 CFU/ml. One hour after inoculation, animals were dosed subcutaneously (SC) with vehicle or various dose regimens of the test drug. At 0 h and 24 h, cohorts of animals were euthanized (CO_2 asphyxiation) and the thigh was aseptically removed. The thigh was weighed and placed into 10 ml sterile saline and homogenized. For the majority of strains, dilutions of the homogenate were plated on tryptic soy agar plates (Teknova, Hollister, CA) which were incubated overnight. In the case of the PSSP strains, dilutions of the homogenate were plated on tryptic soy blood agar plates, which were incubated overnight in a CO_2 incubator. In all cases, the bacterial burden was expressed as $\log_{10}$ CFU/g of thigh weight.

Pharmacokinetic studies. A separate single-dose pharmacokinetic study of TD-1792 was performed with neutropenic NSA mice infected in the thigh with MRSA 33591. Infected animals were dosed, at 1 h postinoculation, with 3 mg/kg of TD-1792 SC. Groups of three mice were sacrificed at predose and at 0.033, 0.083, 0.25, 0.5, 1, 2, 4, 8, and 24 h postdose.

Animals were euthanized with CO_2, and blood samples were collected by cardiac puncture. Drug concentrations were assayed by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). Samples (100 μl) were extracted using solid-phase extraction and were injected on a Discovery HS C_{18}, 5-μm high-performance liquid chromatography (HPLC) column (2.1 × 50 mm) with a flow rate of 0.6 ml/min. Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B consisted of 0.1% formic acid in acetonitrile. The gradient elution was started at 5% B and ramped to 16% B and 95% B at 2.9 and 3.1 min, respectively, and held for 0.4 min. The HPLC column was reequilibrated for 1.3 min at 5% B. The mass spectrometer (Sciex API4000; Applied Biosystems, Foster City, CA) was operated in positive-ion multiple-reaction monitoring mode. TD-1792 was monitored using mass transitions of 992.4/952.8. D5-TD-1792 was used as the internal standard and monitored using mass transitions of 997.1/955.5. The dynamic range of the assay was 10.0 to 10,000 ng/ml. The percent accuracy of the standard curve ranged from 96.0 to 104%. The percent coefficient of variation (%CV) ranged from 1.0 to 9.2%.

Experimental treatments. The murine experiments consisted of a single-dose study and multiple dose-fractionation studies. In the single-dose study, TD-1792 was administered to infected mice over a dose range of 0.03 to 10 mg/kg SC. In dose-fractionation studies, the total 24-h dose of TD-1792 of 0.1, 0.3, or 1 mg/kg, SC, was administered either as a single dose (every 24 h) or as two (every 12 h), three (every 8 h), or four (every 6 h) divided doses. As a comparator, vancomycin was tested for efficacy in MRSA 33591-infected mice. Vancomycin was administered every 12 h, and total daily doses in the range of 1 to 300 mg/kg, SC, were tested.

Data analysis. (i) Pharmacodynamic analysis. Dose-response curves were fit with a four-parameter logistic equation using GraphPad Prism, version 3.00 for Microsoft Windows (GraphPad Software, San Diego, CA). The equation used was: $Y = \text{Min} + (\text{Max} - \text{Min})/(1 + 10^{[\log \text{ED50} - X] \times \text{Hillslope}})$, where X is the logarithm of dose, Y is the response ($\log_{10}$ CFU/g), with Y starting at a minimum (Min) (fixed to the 24-h vehicle control response) and asymptotically approaching a maximum (Max) with a sigmoidal shape, and ED_{50} is defined as the dose required to produce 50% of the maximum response. ED_{50} estimates were expressed as means with 95% confidence intervals (CI). Two-tailed Student's *t* test was used to compare ED_{50} estimates between treatments. A *P* value of <0.05 was

TABLE 1 Point estimates of the TD-1792 dose required to achieve various PD endpoints for each Gram-positive bacterial strain studied in the neutropenic murine thigh model

Strain	MIC[a] (μg/ml)	Doses (mg/kg, SC) of TD-1792 required to achieve various PD endpoints[b]				
		ED$_{stasis}$	Emax	ED$_{1\text{-logkill}}$	ED$_{2\text{-logkill}}$	ED$_{3\text{-logkill}}$
MSSA 29213	0.015 (1)	0.44	2.38	0.79	1.48	ND
MRSA (ATCC 33591)	0.03 (1)	0.53	1.67	1.11	10	ND
VISA (HIP5836)	0.12 (4)	1.34	3.17	2.55	5.33	10
MSSE (SU03)	0.008 (2)	0.069	3.79	0.17	0.40	1.07
MRSE (MED 820)	0.03 (2)	0.03	2.15	0.13	0.60	ND
PSSP (MED 35)	0.004 (0.5)	0.23	4.66	0.43	0.71	1.38
PSSP (MED 1119)	0.002 (0.5)	0.031	4.60	0.049	0.07	0.122
S. pyogenes (MED 2040)	0.0005 (0.25)	0.72	3.79	0.98	1.56	2.47

[a] Values in parentheses denote the corresponding MICs for vancomycin.
[b] Refer to Materials and Methods for the definition of PD endpoints. ND, not determined; endpoint was not achieved at the doses tested.

considered to be statistically significant. The $\log_{10}$ stasis dose (ED$_{stasis}$) was defined as the dose producing no net change in the thigh bacterial burden compared to the pretreatment bacterial burden. A 1-$\log_{10}$ kill dose (ED$_{1\text{-log kill}}$) was defined as the dose required to produce a decrease in bacterial burden of 1 log CFU/g from pretreatment controls. Similarly, 2- and 3-$\log_{10}$ kill doses were defined as the doses required to produce a decrease in bacterial burden of 2 and 3 $\log_{10}$ CFU/g, respectively, from pretreatment controls.

(ii) Pharmacokinetic analysis. Pharmacokinetic models were fit to pooled plasma exposure data using weighted nonlinear regression in S-ADAPT 1.53 software (4). Model discrimination was accomplished according to the rule of parsimony based on the Akaike information criterion (1). Weighting of the plasma data was based on the reciprocal of the estimated observation variance, which was predicted as a function of the fitted concentration, using a constant (additive) plus proportional residual error model. Parameter estimates from the final pharmacokinetic model were used to simulate concentration-time profiles for various dosing regimens for mice studied in the single dose and dose-fractionation pharmacodynamic (PD) studies. Measures of exposures were generated from these simulated profiles.

(iii) Pharmacokinetic-pharmacodynamic analysis. Data obtained from mice infected with MRSA ATCC 33591 were used for the PK-PD analysis. The PK-PD measures chosen for evaluation were the percentage of the 24-h period of study for which the total-drug plasma concentration remained above the MIC (%Time>MIC), the ratio of the 24-h total-drug area under the concentration-time profile to the MIC (AUC/MIC ratio), and the ratio of the maximum total-drug plasma concentration (Cmax) to the MIC (Cmax/MIC ratio).

The %Time>MIC was obtained by calculating the percentage of time the total-drug plasma concentration was above the nominal MIC (0.031 μg/ml) in a 24-h period. Given the short half-life of TD-1792 in mice, the AUC from 0 h to infinity (AUC$_{0\text{-inf}}$) was assumed to be equal to the AUC from 0 to 24 h (AUC$_{0\text{-}24}$). AUC$_{0\text{-inf}}$ was calculated using the equation AUC$_{0\text{-inf}}$ = D/CLt, where D is the dose in mg/kg and CLt is the clearance of TD-1792. Cmax for each dosing regimen was calculated from the simulated concentration-time data. AUC$_{0\text{-inf}}$ and Cmax were divided by the MIC value to obtain the AUC/MIC and Cmax/MIC ratios, respectively.

Using S-ADAPT 1.53 software, data were modeled using a Hill-type function, with nonlinear least-squares regression, which described the relationships between the change in $\log_{10}$ CFU/g from baseline and the PK-PD measure of interest (AUC/MIC ratio, Cmax/MIC ratio, and %Time>MIC). The PK-PD targets based on such relationships which were associated with no change in $\log_{10}$ CFU/g (net bacterial stasis) or a 1 $\log_{10}$ CFU/g reduction from baseline (1 log kill) were also calculated.

RESULTS

***In vitro* activity of TD-1792.** As shown in Table 1, the MIC values for TD-1792 against the collection of the staphylococcus and streptococcus isolates ranged from 0.0005 to 0.12 μg/ml. The MIC

range for vancomycin against these strains was 0.25 to 8 μg/ml. Against MRSA ATCC 33591, the MIC for TD-1792 (MIC = 0.03 μg/ml) was 32-fold lower than that for vancomycin (MIC = 1 μg/ml). In the presence of mouse serum/albumin, the MIC of TD-1792 increased from 0.015 to 0.06 μg/ml.

Protein binding. TD-1792 exhibited concentration-independent protein binding in mouse and human plasma that ranged from 48% to 54% and 44% to 49%, respectively.

Single-dose pharmacokinetics of TD-1792 in infected neutropenic mice. TD-1792 mouse plasma concentration-time data following a single SC dose of 3 mg/kg was fitted using a two-compartment model (one central and one peripheral) with zero-order absorption (approximating the subcutaneous injection) and first-order elimination. The model-fitted plasma concentration-time profile overlaid with the observed data is shown in Fig. 2. The correlation coefficient (r^2) for the relationship between the observed and fitted concentrations was 0.866, indicating that the data were well described by the model.

The pharmacokinetic parameter estimates for the clearance out of the central compartment, the volume of the central compartment, the distributional clearance between the central
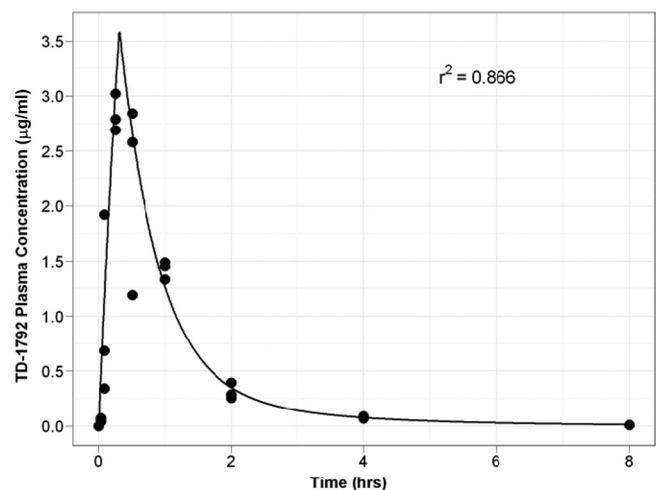

FIG 2 Observed and fitted single-dose (3 mg/kg, SC) concentration-versus-time pharmacokinetic profile for TD-1792 in neutropenic mice infected in the thigh with MRSA ATCC 33591. Abscissa shows the time (hours), and ordinate shows the plasma drug concentration (μg/ml) (n = 3 per group).
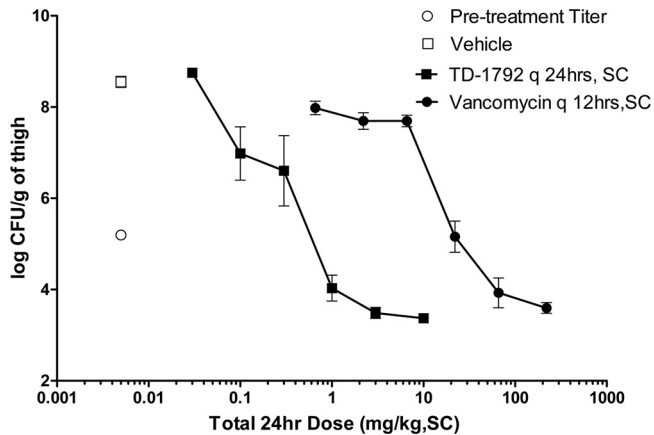
FIG 3 Efficacy of TD-1792 and vancomycin against MRSA ATCC 33591 in the neutropenic murine thigh model. The abscissa shows the total 24-h dosage in mg/kg, SC, and the ordinate shows the thigh bacterial burden in log$_{10}$ CFU/g. Vehicle and TD-1792 were dosed every 24 h, whereas vancomycin was dosed every 12 h. Data are expressed as means ± standard deviations (SD) (*n* = 5 per group).
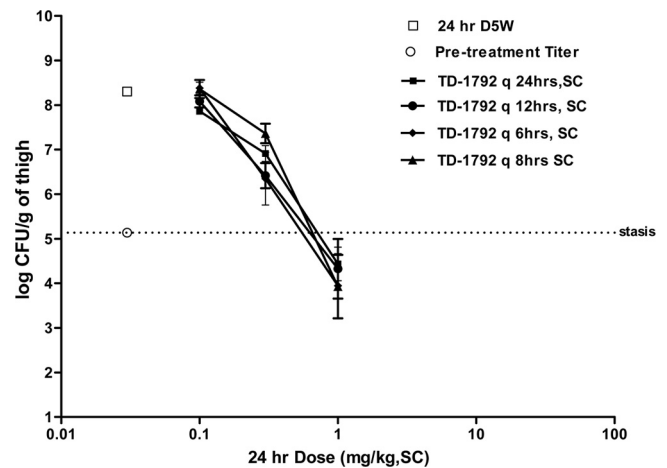

FIG 4 Effect of TD-1792 against MRSA ATCC 33591 on thigh bacterial burden in mice dosed with various total 24-h doses of TD-1792 (0.03 to 1 mg/kg, SC) administered in one, two, or four divided fractions. The abscissa shows the total 24-h dosage in mg/kg, SC, and the ordinate shows the thigh bacterial burden in log$_{10}$ CFU/g. Data are expressed as means ± SD (*n* = 5 to 10).

compartment and the peripheral compartment, and the volume of the peripheral compartment were 0.907 liters/kg/h, 0.659 liters/kg, 0.144 liters/kg/h, and 0.255 liters/kg, respectively. The alpha half-life estimate in mice was 0.41 h, and the terminal half-life was 1.7 h.

Pharmacodynamic effects of TD-1792 against multiple Gram-positive organisms in the neutropenic murine thigh model. TD-1792, dosed SC, produced a dose-dependent reduction of thigh bacterial burden when tested against multiple Gram-positive pathogens in the neutropenic murine thigh model. A summary of the point estimates of the TD-1792 dose required to achieve various PD endpoints for each strain studied is provided in Table 1. The magnitude of kill was strain dependent. TD-1792 produced a maximal of approximately 1- to 2-log$_{10}$ kill against MRSA ATCC 33591, MSSA ATCC 29213, and MRSE MED 820 and ≥3-log$_{10}$ kill against VISA HIP 5836, MSSE SU03, PSSP MED35, PSSP MED1119, and *S. pyogenes* MED 2040 strains. The potency of TD-1792, assessed by the ED$_{1-log kill}$, ranged from 0.049 to 2.55 mg/kg, SC.

The efficacy of TD-1792 was compared to that of vancomycin against MRSA ATCC 33591 in the neutropenic thigh model infection. The dose-response curves of the two drugs are shown in Fig. 3. Against this organism, vancomycin produced a dose-dependent reduction in the thigh bacterial burden with ED$_{stasis}$ and ED$_{1-logkill}$ values of 23.4 and 54.6 mg/kg, respectively, which were 44- and 49-fold higher than those for TD-1792. The maximum kill (Emax) was 1.67 (0.71 to 2.01) log$_{10}$ CFU/g for TD-1792 and 1.46 (1.16 to 1.79) log10 CFU/g for vancomycin, and these values were not significantly different from each other.

Effects of dose fractionation on the efficacy of TD-1792 in the murine neutropenic thigh model. Figure 4 shows the effects of TD-1792 against MRSA ATCC 33591 on the bacterial burden in mice dosed with total 24-h doses of TD-1792 ranging from 0.03 to 1 mg/kg, SC, administered in one, two, or four divided fractions. The pretreatment bacterial burden was 5.13 ± 0.06 log$_{10}$ CFU/g (*n* = 10 mice). In vehicle control-treated animals, the bacterial burden was 8.30 ± 0.01 log$_{10}$ CFU/g (*n* = 10). TD-1792 produced a dose-dependent reduction of the thigh bacterial burden in all

dose-fractionation groups (Fig. 4). At any given dose, there was no significant difference (*P* > 0.05) in efficacy among the different dose-fractionation groups. The ED$_{stasis}$ of TD-1792 was 0.71, 0.61, 0.64, and 0.54 mg/kg in groups treated every 24, 12, 8, and 6 h, respectively.

PK-PD analysis. Relationships between each of the three PK-PD indices and change in log$_{10}$ CFU/g thigh bacterial burden from baseline were evaluated using a Hill-type function (Fig. 5). The AUC/MIC ratio was most predictive of efficacy (r^2 = 0.826) compared to the Cmax/MIC ratio (r^2 = 0.715) or %Time>MIC (r^2 = 0.749). The equation for the function describing the relationship between efficacy and the AUC/MIC ratio is as follows: change in log$_{10}$ CFU/g = 3.25 − (5.12 × AUC/MIC$^{1.83}$)/(15.6$^{1.83}$ + AUC/MIC$^{1.83}$).

The PK-PD model parameter estimates and the 95% CI for these estimates are shown in Table 2.

The magnitudes of the AUC/MIC ratios associated with net bacterial stasis, a 1 log$_{10}$ CFU/g reduction from baseline, and near maximal effect (EC$_{90}$) were estimated and are summarized in Table 3.

DISCUSSION

TD-1792 is a novel antibiotic that is chemically a covalently linked heterodimer of a glycopeptide (vancomycin) and a cephalosporin moiety (14). Its two component moieties simultaneously target D-Ala-D-Ala-containing peptidoglycan precursors and the active site of penicillin-binding protein (15). We have hypothesized that simultaneous and/or sequential binding of the vancomycin component to the nascent peptidoglycan substrate presents a high effective concentration of the cephalosporin component at the active site of both PBP2 and PBP2a, thereby conferring high potency to the molecule. TD-1792 is a bactericidal drug that exerts potent antibacterial activity against clinically relevant Gram-positive pathogens, including multidrug-resistant organisms such as MRSA and VISA, and its activity cannot be reproduced by the individual monomer tested either alone or in combination (10, 13). In the present study, we evaluated the PK and PD properties of TD-1792 in the neutropenic murine thigh model to assess its *in*
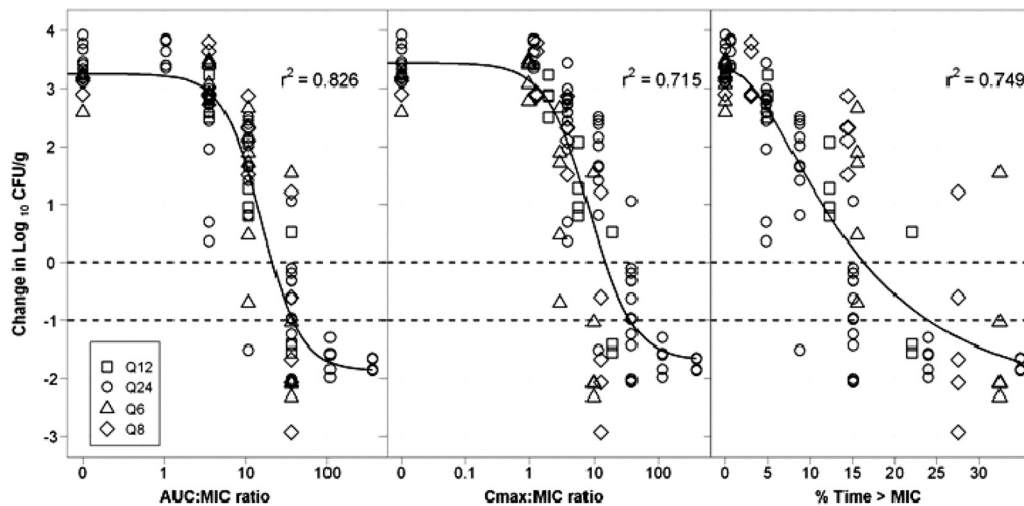
FIG 5 Relationship between AUC/MIC ratio (left), Cmax/MIC ratio (middle), %Time>MIC (right), and the change in $\log_{10}$ CFU/g from pretreatment values. Each point represents data from one mouse. The fitted line represents the Hill-type function.

vivo potency and to determine the PK-PD index and the magnitude of this index associated with efficacy.

In vitro susceptibility studies demonstrated that TD-1792 was active and potent against all Gram-positive strains that were tested, including those which are highly resistant to oxacillin (MRSA and MRSE strains) and demonstrate intermediate susceptibility to vancomycin (VISA HIP 5836). The MIC of TD-1792 against MRSA ATCC 33591 was shifted 4-fold in the presence of mouse serum, from 0.015 to 0.06 μg/ml. This observed shift was slightly but not meaningfully greater than the 2-fold shift predicted from the magnitude of protein binding (~50%) of the drug.

TD-1792 displayed potent antibacterial efficacy *in vivo* against all the organisms tested. At doses of 10 mg/kg and lower, TD-1792 produced ≥1-log kill against all organisms, consistent with its potent *in vitro* bactericidal activity. The *in vivo* rank order of potency of TD-1792 versus the different strains was somewhat consistent with the *in vitro* potencies. For example, TD-1792's MIC against VISA HIP 5836, which was 4-fold-higher than that against MRSA ATCC 33591, also translated to higher ED_{stasis} and $ED_{1-3\ log\ kill}$ doses *in vivo*. Furthermore, TD-1792's 32-fold-greater *in vitro* potency over vancomycin against the MRSA ATCC 33591 was reflected in the >40-fold improvement *in vivo* potency based on the data from the neutropenic murine thigh model.

TABLE 2 Parameter estimates of the model describing the relationship between the AUC/MIC ratio and the efficacy for TD-1792 against MRSA ATCC 33591 in the neutropenic murine thigh model of infection[a]

Parameter	Estimate	95% CI
E_{con}	3.25	2.87–3.63
E_{max}	5.12	4.23–6.00
Hill	1.83	1.12–2.54
EC_{50}	15.6	11.3–19.9

[a] E_{con} is the change in $\log_{10}$ CFU/g from the baseline after 24 h when no drug is administered; E_{max} is the maximum change in $\log_{10}$ CFU/g from the baseline at 24 h after administering TD-1792; the AUC/MIC ratio is the PK-PD index of interest; EC_{50} is the value for the AUC/MIC ratio at which there is half-maximal change in $\log_{10}$ CFU/g; and Hill is the Hill's coefficient of sigmoidicity.

It is now well recognized that achieving optimal efficacy for any given antibacterial agent requires a careful understanding of its PK-PD properties (3, 7, 8, 11). Dose-fractionation efficacy studies provide a useful way to elucidate PK-PD relationships for antibacterials (8). Using this approach, we demonstrated that the antibacterial potency of TD-1792 in the neutropenic thigh model was unaffected by changing the dosing interval, consistent with total exposure serving as the principal determinant of efficacy. Additionally, PK-PD analysis of the data demonstrated that the AUC/MIC ratio was the PK-PD measure most predictive of reduction in bacterial burden. Although the PD effects were also reasonably well correlated with %Time>MIC and Cmax/MIC, visual examination of the plots revealed a larger degree of scatter of observed data around fitted functions for these PK-PD indices. If one examines the plot for %Time>MIC in Fig. 5, one can see that the regimens, differentiated by interval, stack on top of one another. In contrast, for the AUC/MIC ratio plot, the symbols for the regimen are dispersed and not layered as they appear to be for %Time>MIC.

Given the similar magnitudes of protein binding in mouse and human plasma, total-drug AUC/MIC ratios associated with various levels of bacterial reduction from baseline for TD-1792 are reported herein. AUC/MIC ratios of 21.1 and 37.2 were associated with net bacterial stasis and a 1 $\log_{10}$ CFU/g reduction from baseline, respectively. Additionally, since previous PK-PD analyses using data from both patients with complicated skin and skin structure infections (cSSSI) and neutropenic murine infection models have demonstrated the magnitude of the PK-PD measure required for efficacy in infected patients to be similar to that re-

TABLE 3 TD-1792 AUC/MIC ratios associated with net bacterial stasis, 1 $\log_{10}$ CFU/g reduction from baseline, and EC_{90} against MRSA ATCC 33591 in the neutropenic murine thigh animal model

Endpoint	Estimate	95% CI
Net bacterial stasis	21.1	17.0–25.3
1 $\log_{10}$ CFU/g reduction from baseline	37.2	26.9–47.5
EC_{90}[a]	51.8	29.9–73.8

[a] EC_{90}, value of the AUC/MIC ratio at which there is 90% of maximal effect.

quired for net bacterial stasis or a 1-log$_{10}$ CFU/g reduction from baseline in the neutropenic murine thigh infection model (2). Monte Carlo simulations using such PK-PD targets served to provide a reasonable minimum exposure threshold upon which to base early dose selection decisions for patients with cSSSI (5). Although plasma concentrations were well described by the murine PK model, the single-dose nature of the PK study may have resulted in some degree of underestimation of the above-described AUC/MIC ratio targets. The assumption of linearity in clearance across all doses evaluated in the PK-PD study should, therefore, be confirmed in a future study.

Overall, the data generated in the current studies using a neutropenic murine thigh infection model demonstrated that TD-1792 is a potent and efficacious antibiotic *in vivo* against a collection of susceptible and resistant Gram-positive organisms. PK-PD evaluations for MRSA demonstrated that the AUC/MIC ratio is the best predictor of efficacy for this drug. The integration of PK-PD targets for efficacy, based on PK-PD relationships derived using data from such *in vivo* infection models, together with data from phase 1 pharmacokinetic studies, provides informative dose selection support for phase 2 studies (5). The results of PK-PD target attainment analyses for TD-1792, which were based on the AUC/MIC ratio targets described herein, were used to support dose selection for a phase 2 study of patients with cSSSI. Data from this study subsequently showed that a 2-mg/kg intravenous dose of TD-1792 administered once daily was well-tolerated and non-inferior to vancomycin in the treatment of cSSSI caused by Gram-positive bacteria (17). TD-1792 has the potential to be a valuable addition to the therapeutic armamentarium for the treatment of Gram-positive infections.

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