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Population Pharmacokinetic/Pharmacodynamic Analysis of the Bactericidal Activities of Sutezolid (PNU-100480) and Its Major Metabolite against Intracellular *Mycobacterium tuberculosis* in *Ex Vivo* Whole-Blood Cultures of Patients with Pulmonary Tuberculosis

Tong Zhu,^a Sven O. Friedrich,^b Andreas Diacon,^b Robert S. Wallis^{a*}

Pfizer, Groton Connecticut, USA^a; Division of Medical Physiology, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, South Africa^b

Sutezolid (PNU-100480 [U-480]) is an oxazolidinone antimicrobial being developed for the treatment of tuberculosis. An active sulfoxide metabolite (PNU-101603 [U-603]), which reaches concentrations in plasma several times those of the parent, has been reported to drive the killing of extracellular *Mycobacterium tuberculosis* by sutezolid in hollow-fiber culture. However, the relative contributions of the parent and metabolite against intracellular *M. tuberculosis* *in vivo* are not fully understood. The relationships between the plasma concentrations of U-480 and U-603 and intracellular whole-blood bactericidal activity (WBA) in *ex vivo* cultures were examined using a direct competitive population pharmacokinetic (PK)/pharmacodynamic 4-parameter sigmoid model. The data set included 690 PK determinations and 345 WBA determinations from 50 tuberculosis patients enrolled in a phase 2a sutezolid trial. The model parameters were solved iteratively. The median U-603/U-480 concentration ratio was 7.1 (range, 1 to 28). The apparent 50% inhibitory concentration of U-603 for intracellular *M. tuberculosis* was 17-fold greater than that of U-480 (90% confidence interval [CI], 9.9- to 53-fold). Model parameters were used to simulate *in vivo* activity after oral dosing with sutezolid at 600 mg twice a day (BID) and 1,200 mg once a day (QD). Divided dosing resulted in greater cumulative activity ($-0.269 \log_{10}$ per day; 90% CI, -0.237 to $-0.293 \log_{10}$ per day) than single daily dosing ($-0.186 \log_{10}$ per day; 90% CI, -0.160 to $-0.208 \log_{10}$ per day). U-480 accounted for 84% and 78% of the activity for BID and QD dosing, respectively, despite the higher concentrations of U-603. Killing of intracellular *M. tuberculosis* by orally administered sutezolid is mainly due to the activity of the parent compound. Taken together with the findings of other studies in the hollow-fiber model, these findings suggest that sutezolid and its metabolite act on different mycobacterial subpopulations.

Mycobacterium tuberculosis resistance to standard first-line drugs (drug-resistant tuberculosis [DR-TB]) is a serious and growing global health threat, causing at least 444,000 new tuberculosis (TB) cases and 150,000 deaths annually (1). Oxazolidinone antimicrobials are increasingly viewed as candidates for inclusion in new regimens for DR-TB, as they have a distinct mechanism of action (binding to the 23S ribosome) without cross-resistance to current drugs. Linezolid is the only currently licensed oxazolidinone. Sutezolid (PNU-100480 [U-480]) is a thiomorpholinyl analog of linezolid with superior efficacy against *M. tuberculosis* in the hollow-fiber, mouse, and whole-blood models (2–4). Time-dependent killing of *M. tuberculosis* by sutezolid has been reported in whole blood and hollow fibers (2, 3).

Orally administered sutezolid is rapidly oxidized *in vivo* to an active sulfoxide metabolite (PNU-101603 [U-603]), which then undergoes renal excretion. The concentrations of U-603 achieved in human plasma are severalfold greater than those of the parent. Some studies have reported similar MICs for both U-480 and U-603 (0.25 $\mu\text{g/ml}$) for reference strains and clinical isolates, regardless of the method of testing (5, 6); however, others have reported lower median MIC values ($\leq 0.062 \mu\text{g/ml}$) for the parent when clinical isolates are tested in liquid culture (6).

The relative contributions of the parent and metabolite to killing of mycobacteria appear to differ according to cellular location. Studies in the hollow-fiber model using concentrations of parent and metabolite that are achieved in human plasma have indicated that killing of extracellular mycobacteria by sutezolid is mainly

due to the metabolite (2). In contrast, studies in which sutezolid or its metabolite were added separately to cultures of *M. tuberculosis*-infected mammalian cells have found the parent to be at least 10-fold more potent than the metabolite (7). However, these studies have not examined the activity of sutezolid as it would occur in the cells of patients with tuberculosis, which may differ in immune function and drug metabolism.

The present study used mathematical modeling to examine the relative contributions of U-480 and U-603 to the killing of intracellular *M. tuberculosis* in *ex vivo* cultures measuring whole-blood bactericidal activity (WBA) that were conducted in clinical trial B1171003, the first study of sutezolid in patients with pulmonary tuberculosis.

MATERIALS AND METHODS

Subjects. As previously reported (8), subjects consisted of men and women aged 18 to 65 years with chest radiographs with findings consistent

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Address correspondence to Robert S. Wallis, rswallis@gmail.com.

* Present address: Robert S. Wallis, Aurum Institute, Johannesburg, South Africa.

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with pulmonary tuberculosis, positive sputum acid-fast smears, culture or molecular confirmation of drug-susceptible *M. tuberculosis*, a random blood glucose level of <150 mg/dl, reasonably normal renal and hepatic function, and a willingness to provide written informed consent according to International Conference on Harmonization guidelines. Subjects were either HIV-1 uninfected or HIV-1 infected with CD4 T cell counts of >350/mm³ and not currently receiving antiretroviral therapy. This study received ethical approval from the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee, Cape Town, South Africa, and from Pharma-Ethics, Lyttelton Manor, South Africa.

Treatments. Subjects were randomly assigned to receive sutezolid at 600 mg twice a day (BID; $n = 25$) or 1,200 mg once a day (QD; $n = 25$) or to receive a positive control of fixed-dose combination tablets consisting of isoniazid, rifampin, pyrazinamide, and ethambutol (HRZE; Rifafour e275; $n = 9$). The data for HRZE-treated subjects were not included in the present analysis.

Evaluations. Blood was collected for WBA at the baseline (WBA₀) and for pharmacokinetic (PK) and WBA determinations on days 13 and 14 (at 0, 1, 2, 3, 6, 8, and 12 h postdosing). Plasma was separated immediately after collection and stored at -20°C for PK determinations. Total plasma concentrations of PNU-100480 and PNU-101603 were determined using a validated high-pressure liquid chromatography-tandem mass spectrometry method by Advion BioServices (Ithaca, NY), as previously described (9).

WBA was determined as previously described (9). Briefly, an *M. tuberculosis* H37Rv stock was prepared in mycobacterial growth indicator tubes (MGITs; Becton, Dickinson, Sparks, MD) containing oleic acid, albumin, dextrose, and catalase (OADC; Becton, Dickinson) and polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA; Becton, Dickinson). For each batch of stock, a titration experiment described the relationship between the inoculum volume and MGIT time to positivity (TTP). Whole-blood cultures consisted of heparinized blood, an equal volume of RPMI 1640 tissue culture medium (Highveld Biological, Lyndhurst, South Africa), and bacilli of the volume of the *M. tuberculosis* H37Rv stock identified by the titration curve to have an MGIT TTP of 5.5 days. Direct inoculation of this volume into an MGIT culture served as a growth control. Whole-blood cultures were incubated with slow constant mixing for 72 h, after which cells were sedimented, the liquid phase was removed, and blood cells were disrupted by hypotonic lysis. Bacilli were recovered and inoculated into MGIT cultures. The log change in viability was calculated as $\log(\text{final}) - \log(\text{initial})$, where final and initial were the volumes corresponding to the TTPs of the completed whole-blood culture and its inoculum, respectively, on the basis of the titration curve of the stock. Results were expressed as the log change per day ($\Delta\log/d$) of whole-blood culture. The cumulative WBA over 24 h was calculated as the area under the concentration-time curve from time zero to 24 h of values measured at discrete time points, using the trapezoidal method. Results were expressed as $\Delta\log/d \cdot d$, or simply as the log change.

Mathematical modeling. A population PK/pharmacodynamic (PD) model was developed to simultaneously describe the relationship between the concentrations of U-480 and U-603 observed in plasma and the bactericidal activity observed in whole-blood cultures. The relationship between drug concentration and bactericidal activity was examined using a 4-parameter sigmoid curve, on the basis of previous observations that killing was concentration dependent at low concentrations but did not increase further as a maximal response (I_{\max}) was approached (3). The activities of U-480 and U-603 were assumed to be competitive, on the basis of previous *in vitro* observations that the addition of U-603 to optimal concentrations of U-480 did not further increase intracellular drug activity (10). The equations used to describe bactericidal activity were as follows: $\text{WBA} = \text{WBA}_0 - \{I_{\max}[(P + M)/(P + M + 1)]\}$, $P = (C_{480}0.5)/IC_{50}480\gamma_{480}$, and $M = (C_{603}0.5)/IC_{50}603\gamma_{603}$, where WBA is the model-predicted effect; WBA₀ is the baseline effect; I_{\max} is the maximum effect; IC₅₀480 is the 50% inhibitory concentration of U-480, or the concentration of U-480 for 50% of the maximum effect; IC₅₀603 is the 50% inhib-

TABLE 1 Final parameter estimates and 90% CIs

Parameter	Estimate	90% CI
Fixed effects		
WBA ₀ ($\Delta\log/d$)	0.182	0.154–0.209
I_{\max} ($\Delta\log/d$)	0.597	0.559–0.636
IC ₅₀ 480 (ng/ml)	70.4	56.0–84.9
IC ₅₀ 603 (ng/ml)	1,200	839–2,960
γ_{480}	2.25	1.7–3.2
γ_{603}	(0.94) ^a	
Random effects		
WBA ₀ (%)	27.4	19.4–35.2
Sigma ^b	0.096	0.075–0.114

^a The initial estimate for γ_{603} (0.94) could not be distinguished from 1 and was omitted from the final model.

^b Sigma, residual error.

itory concentration of U-603, or the concentration of U-603 for 50% of the maximum effect; γ_{480} is the shape factor (Hill coefficient) of the concentration-effect relationship for U-480, γ_{603} is the shape factor (Hill coefficient) of the concentration-effect relationship for U-603, C_{480} is the observed plasma concentration of U-480, and C_{603} is the observed plasma concentration of U-603.

The plasma concentrations of U-480 and U-603 were adjusted by a factor of 0.5 to account for the dilution of blood with tissue culture medium in the whole-blood cultures.

The model was developed using the \$PRED subroutine and FOCE-I in the NONMEM (version 7.1.2) program (11). Intersubject variability was tested for all model-estimated parameters alone or in combination. Residual variability was described with an additive error model. Model adequacy was assessed by goodness-of-fit plots and visual predictive checks ($n = 1,000$), using the R package (version 2.12.2) (12). The precision of the parameter estimates was obtained by nonparametric bootstrap analysis ($n = 1,000$), using the PsN (version 3.2.12) program (13). The WBA time course was simulated for U-480, U-603, and U-480–U-603 on the basis of final model parameters and median plasma concentration-time profiles.

RESULTS

A total of 690 PK determinations and 345 WBA determinations from 50 subjects were analyzed. The median ratio of the U-603/U-480 plasma concentrations was 7.1 (range, 1 to 28). High ratios mainly occurred at late time points in the dosing interval. The wide range of values facilitated modeling of the relative contributions of parent and metabolite to overall activity. The model was solved iteratively to determine the parameter values that most closely predicted actual WBA results. Final parameter estimates are indicated in Table 1. The concentration of U-603 required for a half-maximal effect (IC₅₀603) was 17-fold greater than that for U-480 (90% confidence interval [CI], 9.9 to 53). The value of γ (the shape factor of the concentration-effect relationship) was determined for both U-480 and U-603. Its value differed significantly from 1 only for U-480. Intersubject variability was tested for IC₅₀480, IC₅₀603, and WBA₀; however, only intersubject variability in WBA₀ remained significant in the final model. The relatively narrow confidence intervals (Table 1) supported the adequacy of the fit of the model. Diagnostic plots (Fig. 1) did not indicate any systematic errors in the model. The eta shrinkage was 20.87%. A visual predictive check (Fig. 2) revealed an adequate correspondence of the observed and predicted values throughout the dosing interval.

The concentration-activity relationships predicted by the

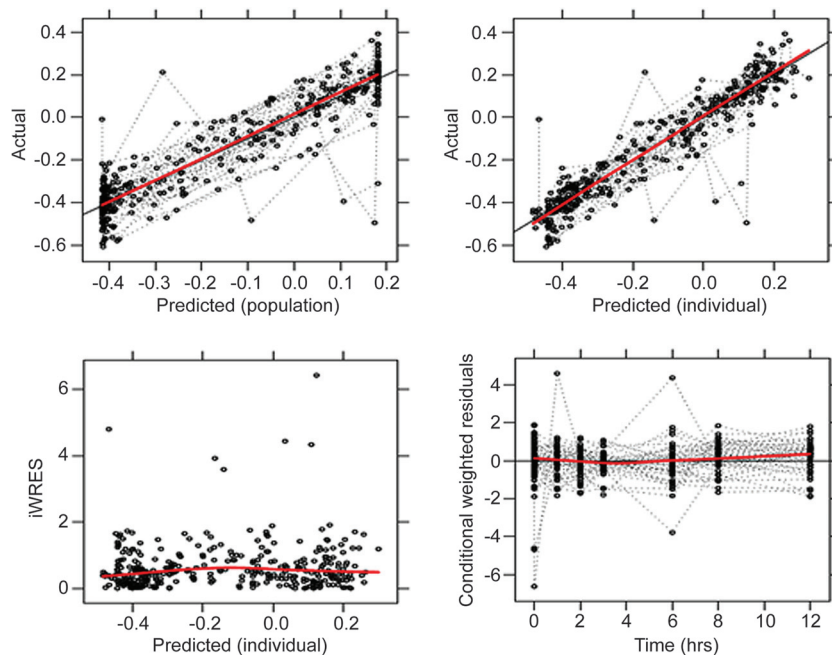


FIG 1 Diagnostic plots to evaluate goodness of fit. iwRES, individual weighted residuals. Actual and predicted values indicate the log change in mycobacterial viability per day ($\Delta\log/d$) of whole-blood culture.

model for sutezolid (U-480) and its metabolite (U-603) on the basis of the fixed parameter estimates in Table 1 are illustrated in Fig. 3. Compared to the curve for U-603, the curve for U-480 was shifted to the left and had a steeper slope. The mean apparent intracellular MIC (the extracellular concentration required for intracellular bacteriostasis) for U-480 was 0.05 $\mu\text{g/ml}$, whereas that for U-603 was 0.55 $\mu\text{g/ml}$, which was 11-fold greater.

The model was then used to simulate mycobactericidal activity *in vivo* after oral dosing with sutezolid at 600 mg BID and 1,200 mg QD, on the basis of median plasma drug concentrations from the recently completed phase 2a trial (8). Unlike the results reported in that trial, however, these reflect drug concentrations and effects as they occur *in vivo* without artifacts resulting from diluting blood with tissue culture medium *in vitro*. Results are shown in Fig. 4. The upper panels indicate activity at discrete time points. Cumulative activity, calculated as the integral over time of values observed at discrete time points, is shown in the lower panels. These take the form of a time-kill curve, converting a static kill

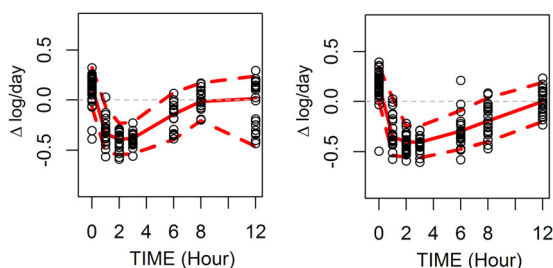


FIG 2 Visual predictive check of model. (Left) Dosing at 600 mg BID; (right) dosing at 1,200 mg QD. Symbols indicate individual observations; solid lines, model predictions; dashed lines, 5th to 95th prediction intervals. The wide confidence interval at 12 h in the 600-mg-BID arm was due to a delay of up to 1 h postdosing in obtaining the 12-h specimen from 7 subjects.

model into a dynamic one. A potential shortcoming of this approach is that postantibiotic effects (PAEs) are not represented; however, studies with linezolid suggest a short PAE (4 h) when oxazolidinones are tested against *M. tuberculosis* (14).

Examination of the cumulative intracellular activity of sutezolid using this approach revealed that divided dosing of sutezolid produced a greater cumulative effect ($-0.269 \log_{10}$ per day; 90% CI, -0.237 to $-0.293 \log_{10}$ per day) than single daily dosing ($-0.186 \log_{10}$ per day; 90% CI, -0.160 to $-0.208 \log_{10}$ per day). U-480 accounted for 78% and 84% of the cumulative daily activity

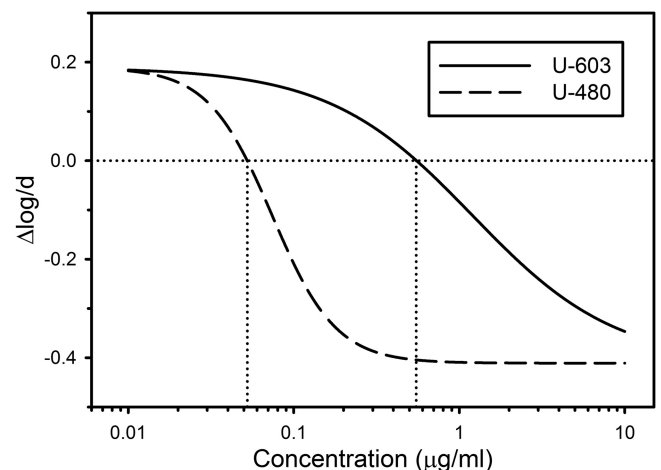


FIG 3 Predicted individual concentration-activity relationships for sutezolid (U-480) and its main metabolite (U-603) against intracellular *M. tuberculosis* on the basis of the fixed-effect estimates of Table 1. The vertical axis indicates the change in mycobacterial viability per day of whole-blood culture, with negative values indicating killing. Dotted lines indicate the concentrations required for intracellular bacteriostasis.

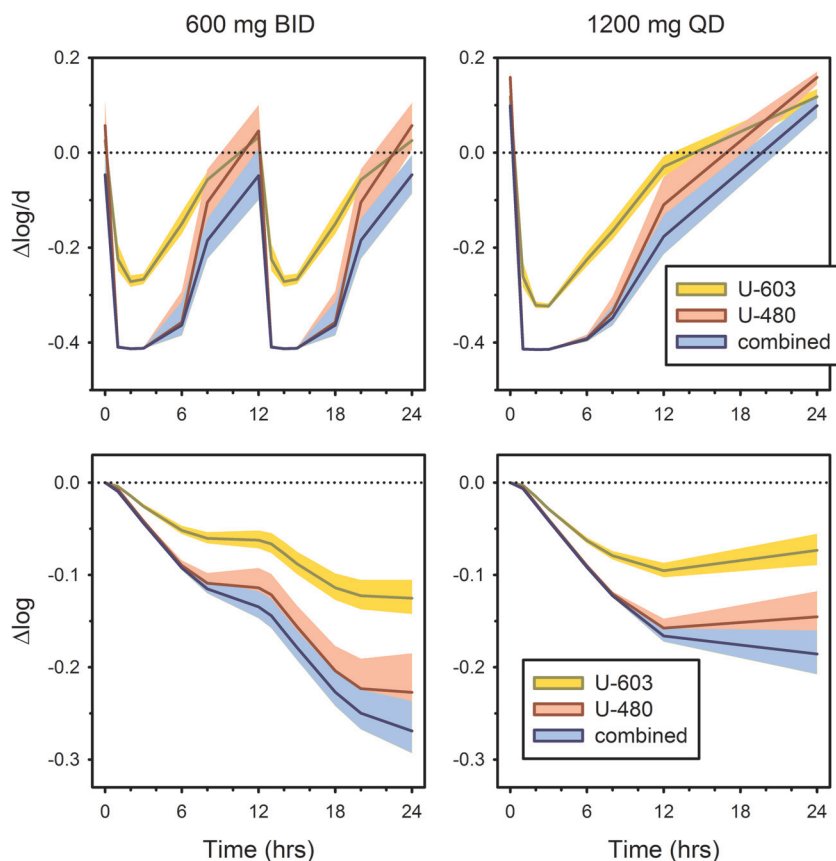


FIG 4 Predicted *in vivo* mycobactericidal activity of U-603 and U-480, individually and in combination, after simulated oral dosing with sutezolid at 600 mg BID and 1,200 mg QD. (Top) Activity at discrete time points; (bottom) cumulative activity, calculated as the integral over time of values at discrete time points. Shading indicates the 90% CI on the basis of PK variability.

when sutezolid was administered at 1,200 mg QD and 600 mg BID, respectively, with the contribution of U-603 becoming significant only at later time points in the dosing interval.

DISCUSSION

This study used a direct PK/PD model to simultaneously determine the relationship between intracellular mycobactericidal activity and plasma concentrations of sutezolid and its major metabolite in patients with pulmonary tuberculosis. The parent (U-480) was found to be 17-fold more potent than its metabolite for killing of intracellular *M. tuberculosis*. Similar results have been reported when the parent and metabolite were added separately to cultures of *M. tuberculosis*-infected mammalian cells (7). The basis of this observation is not known but may reflect differences in intracellular drug accumulation. Approximately 80% of the intracellular activity could be attributed to the parent, despite substantially greater exposures to the metabolite *in vivo*. This finding stands in contrast to extracellular bactericidal activity, which, in the hollow-fiber model, appeared to be mainly due to the metabolite (U-603), due to its higher achieved concentrations. Together, these two observations indicate that drugs that affect sutezolid metabolism may influence outcomes when combined with sutezolid in future TB trials.

Distinct mycobacterial subpopulations exist in patients with tuberculosis, with each subpopulation having distinct biological and clinical significance. Extracellular mycobacteria comprise the

majority of the mycobacterial burden in patients at the time of TB diagnosis, particularly in patients with cavitary pulmonary disease. These bacilli are essential for TB transmission, through the generation by coughing of *M. tuberculosis*-infected aerosol droplets. Because these organisms are, for the most part, actively replicating, this subpopulation is likely responsible for the selection and emergence of drug resistance during treatment. Factors that reduce exposure to U-603 may therefore increase the risk of resistance emerging during sutezolid treatment.

The other mycobacterial subpopulation of importance in tuberculosis is that within tissues and cells. *M. tuberculosis* is readily ingested but not readily killed by host phagocytic cells, resulting in the characteristic pathology of human *M. tuberculosis* infection, the granuloma. Bacilli in tissues exist both within the necrotic centers of granulomas and within intact macrophages near their periphery. Bacillary replication and metabolism in these circumstances are limited by a mycobacterial dormancy response triggered by a lack of oxygen and nutrients. It is thought that the propensity of tuberculosis to relapse despite apparently successful treatment is due to persistence of this subpopulation.

Mycobacteria added to whole-blood cultures rapidly undergo essentially complete phagocytosis by neutrophils and monocytes (15, 16). The extent of mycobacterial growth or killing in the absence of chemotherapy reflects the balance between microbial pathogenicity and host immune mechanisms (15–21). The effects

of administered chemotherapy are reflected in the *ex vivo* whole-blood model as they occur in cells *in vivo*. After standard oral doses, the rank order of activity of anti-TB drugs in the model is rifampin > moxifloxacin > isoniazid > pyrazinamide > ethambutol (16). Standard therapy shows 3 times the activity of current regimens for multidrug-resistant TB. In one small study, the cumulative WBA during TB treatment was superior in those patients whose sputum cultures converted by month 2 (22). Culture status at 2 months is, in turn, an independent predictor of relapse risk (23, 24). The whole-blood model may therefore be best described as an emerging biomarker of drug effects associated with durable TB cure. The observations obtained using this model in the present study suggest that reduced exposure to U-480 due to enhanced metabolism may increase the risk of relapse after treatment with sutezolid.

The oxidative metabolism of sutezolid is not fully understood. CYP3A4 and flavin-containing monooxygenases each contribute 20 to 30% toward its metabolism. CYP3A4 inhibitors or inducers may therefore affect the relative concentrations of sutezolid and its metabolite, thus potentially affecting the two main goals of TB treatment, tissue sterilization and resistance prevention. Specific attention will be warranted to examine the PK-PD relationship of sutezolid in future trials in which it is combined with rifampin, ritonavir, or other drugs that may affect its metabolism.

Finally, this model also enhances our understanding of the PK-PD relationship of sutezolid *in vivo*. Drug concentrations in the *ex vivo* cultures are reduced by half, due to the dilution of blood with tissue culture medium. Modeling removed this artifact, predicting the drug effects at the concentrations achieved *in vivo*. The predicted *in vivo* cumulative activities (-0.269 and -0.186 for BID and QD dosing, respectively) are greater than those previously reported *ex vivo* (-0.142 and -0.089 , respectively) (8). In addition, the difference between divided and single daily dosing is magnified. Longer clinical trials will be required to determine if divided dosing of sutezolid results in accelerated tissue sterilization and a shortened required duration of treatment, as these data would suggest.

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