Br J Clin Pharmacol

Original Research Article

Population pharmacokinetics and pharmacodynamics of linezolid-induced thrombocytopenia in hospitalized patients

Running head; PKPD modelling for linezolid-induced thrombocytopenia

Yasuhiro Tsuji [1, 2] *, Nicholas H. G. Holford [2], Hidefumi Kasai [1, 3], Chika Ogami [1], Young-A Heo [2], Yoshitsugu Higashi [4], Akiko Mizoguchi [5], Hideto To [1], Yoshihiro Yamamoto [4]

1. Department of Medical Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Toyama, Toyama, Japan
2. Department of Pharmacology and Clinical Pharmacology, University of Auckland, Auckland, New Zealand
3. Certara G.K., Tokyo, Japan
4. Department of Clinical Infectious Diseases, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, Toyama, Japan
5. Department of Pharmacy, Sasebo Chuo Hospital, Nagasaki, Japan

* Correspondence: Yasuhiro Tsuji, Ph.D.,

Department of Medical Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama, 930-0194, Japan

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bcp.13262

Tel. / Fax: +81-76-434-7584; E-mail: ytsuji@pha.u-toyama.ac.jp

Word count: Summary = 234; Text = 4,595 (excl. the title page, summary, references, tables, figure legends)

Tables: 4; Figures: 6

Keywords: thrombocytopenia, turnover model, mixture model, linezolid, methicillin-resistant *Staphylococcus aureus*, pharmacokinetics, pharmacodynamics.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

Author contributions

YT contributed to acquisition of data, analyzed and interpreted data, participated in study design and drafted the manuscript. NHGH analyzed and interpreted data and revised the manuscript. HK, YAH and HT contributed to the conception and design, and interpretation of the data. CO contributed to the plasma concentration measurement. YH, AM and YY were the clinical investigators of the trial and responsible for the medical care of trial participants, communication with the research ethics committee, protocol, informed consent, data integrity and reporting. All authors approved the final version to be published.

SUMMARY

Aims: Thrombocytopenia is among the most important adverse effects of linezolid treatment. Linezolid-induced thrombocytopenia incidence varies considerably but has been associated with impaired renal function. We investigated the pharmacodynamic mechanism (myelosuppression or enhanced platelet destruction) and the role of impaired renal function in the development of thrombocytopenia.

Methods: The pharmacokinetics of linezolid were described with a two-compartment distribution model with first-order absorption and elimination. Renal function (RF) was calculated using the expected creatinine clearance. The decrease of platelets by linezolid exposure was assumed to occur with one of two mechanisms in each patient. These mechanisms are inhibition of formation of platelets (PDI) or stimulation of the elimination (PDS) of platelets.

Results: About 50% of elimination is explained by renal CL (normal RF). The population mean estimated plasma protein binding of linezolid was 18% (95% CI 16%-20%) and independent of observed concentrations. The estimated mixture model fraction of patients with platelet count decreased due to PDI was 0.97 (95% CI 0.87-1.00) thus the fraction due to PDS was 0.03. RF had no influence on linezolid pharmacodynamics.

Conclusion: We have described the influence of weight, renal function, age and plasma protein binding on the pharmacokinetics of linezolid. This combined pharmacokinetic, pharmacodynamic and turnover model has identified that the most common mechanism of thrombocytopenia associated with linezolid is inhibition of platelet formation. Impaired RF increases thrombocytopenia by a pharmacokinetic mechanism. Linezolid dose should be reduced in RF.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT; less than 50 words

- Linezolid treatment is associated with thrombocytopenia and is more common with renal function impairment.

WHAT THIS STUDY ADDS

- Weight, renal function and age explain variability in linezolid pharmacokinetics.
- Linezolid thrombocytopenia is most commonly due to myelosuppression rather than platelet destruction.
- Renal impairment increases linezolid exposure. Dose adjustment should reduce thrombocytopenia.

INTRODUCTION

Linezolid is a member of the oxazolidinone class of synthetic antimicrobial agent with a unique mechanism action compared with other existing agents [1]. Linezolid has been used for critical infections including pneumonia, sepsis, wound, skin and soft tissue infections [2-6]. Linezolid has strong antibacterial activity against aerobic Gram-positive *cocci* (GCP), methicillin-resistant coagulase-negative *staphylococci*, vancomycin-resistant *enterococci* and methicillin-resistant *Staphylococcus aureus* (MRSA) [7, 8], and has been approved for use in more than 60 countries. MRSA represents a predominant pathogen associated with serious infections which have become a major therapeutic problem because of high rates of morbidity, mortality, and hospital length of stay [9, 10]. Recent prospective randomized, double-blind trials have compared the use of linezolid compared with vancomycin in for the treatment of adult patients with hospital-acquired or healthcare–associated MRSA pneumonia [11], Gram-positive nosocomial pneumonia [12, 13], and Gram-positive ventilator associated pneumonia [14]. For the treatment of these infections, clinical response was significantly higher with linezolid than vancomycin. On the other hand, three meta analyses have compared linezolid with glycopeptides (including vancomycin) for the treatment of nosocomial MRSA pneumonia [15-17]. The conclusions of all these meta analyses were consistent, showing the clinical cure and survival rates were similar for linezolid and vancomycin. Thus, linezolid is now considered most important for treatment of GCP and MRSA infections.

Linezolid is predominantly metabolized through oxidation of its morpholine ring to an inactive form by non-enzymatic oxidative reactions [1]. Dosing adjustment is considered unnecessary for patients at any stage of renal dysfunction including hemodialysis even though clearance increased by 50% during hemodialysis [18]. However, linezolid concentration are significantly higher in patients with renal function impairment than in those without [19-25]. In general, linezolid is administered at a dose of 600 mg twice daily via oral

and/or intravenous infusion. After the initiation of linezolid treatment, the linezolid trough concentration is assumed to be maintained between 2 to 7 μg/mL [22]. Nukui et al. demonstrated that development of thrombocytopenia occurred more frequently in patients with a linezolid trough concentration of >7.5 μg/mL [26]. It has been recommended that dose should be adjusted based on linezolid concentrations [6, 27].

Thrombocytopenia and anemia are among the most important adverse effects of linezolid treatment. Linezolid-induced thrombocytopenia and anemia incidence varies considerably. Thrombocytopenia has been observed in 7.4% [28] and 11.8 % [29] of linezolid treated patients. Anemia was observed in 4.1% and 38.1% of the same groups. The probability of linezolid-induced thrombocytopenia and anemia of incidence is raised after long-term administration of linezolid [30]. Niwa et al, reported that the incidence of linezolid-induced thrombocytopenia was 17% and that high dose (≥22 mg/kg) was a risk factor [31]. In the previous studies, thrombocytopenia was observed in 32% of patients who received linezolid for more than 10 days [32] which in 32.8% duration of linezolid treatment for more than 14 days [33]. In addition, some group described that linezolid-induced thrombocytopenia and anemia associate in patients with renal function impairment [19, 21, 25, 34] and persistently high linezolid concentrations [23, 35].

The mechanism responsible for linezolid-induced thrombocytopenia have not been clearly delineated. Green et al. [36], reported reversible myelosuppression similar to that seen with chloramphenicol. Bone marrow findings in one patient included abundant megakaryocytes, megaloblastoid white cell maturation and erythroid aplasia. Two studies have reported the use of linezolid exposure to predict thrombocytopenia using a turnover model assuming decreased platelet formation [34, 37]. The turnover model was based on a myelosuppression model formulated by Friberg et al. for chemotherapy induced neutropenia [38]. Sasaki et al. reported using a proliferation cell model that the predicted probability of thrombocytopenia during 14 days of treatment (1,200 mg/day) in patients with creatinine

clearance 10 to 30 mL/min was 32.6 to 51.0% [34]. Boak et al. reported using the stem cell model that linezolid concentration of 8 μg/mL inhibited the synthesis of platelet precursor cells by 50% [37]. Two studies noted that the platelet count reached a nadir at day 15 to 20 post linezolid treatment [34, 37]. A mechanism involving a change in platelet formation would be slow because platelet lifespan is around 8 to 10 days [39].

In contrast, several case reports propose a mechanism involving increased elimination of platelets by a drug-induced immune-mediated destruction [40-42]. This mechanism is based on observations of increased megakaryocytes in bone marrow or drug-related anti platelet antibodies with a rapid onset of platelet decline around 3 to 7 days following initiation of linezolid therapy [40, 42].

In light of this controversy about the mechanism of linezolid induced thrombocytopenia we have investigated the possibility that either myelosuppression or enhanced platelet destruction may be important in an individual patient.

PATIENTS AND METHODS

Ethics

This study was performed in conformity with the Helsinki Declaration after approval by the Ethical Review Board of University of Toyama (approval number: clinical 24-118) and Sasebo Chuo Hospital (approval number: 2012-15). Patient privacy and personal information was handled such that patients could not be identified.

Patients and data Sources

A summary of the data for these patients is presented in Error! Reference source not found.. All patients received linezolid film coated tablet and/or injection (Zyvox®, Pfizer Inc. Tokyo, Japan) for the treatment of GCP or MRSA infections from November 2008 to August

2015 at Sasebo Chuo Hospital, Nagasaki and Toyama University Hospital, Toyama, Japan. The usual dose of linezolid was 10 mg/kg three times a day (pediatric) or 300 mg once a daily to 600 mg twice daily (adult) orally and/or by intravenous drip infusion for 1 to 2 h. Other dosage adjustments of linezolid were performed by physicians' decisions.

Determination of linezolid concentrations

The bulk powder of linezolid for the high-performance liquid chromatography (HPLC) was provided by Pfizer Inc.,. All other reagents were analytical grade and were commercially available. Pharmacokinetic the blood samples of serum and plasma were stored at −80◦C until analysis. Serum and plasma deproteinized with an equivalent volume of acetonitrile and the supernatant after centrifugation was measured by HPLC using the absolute calibration method. The unbound linezolid was obtained by centrifugation of 250 µL of the serum or plasma specimens with a Centrifree ® Ultrafiltration device (Merck Millipore Ltd, Cork, IRELAND) for 30 min at 2000 × g. Total and unbound linezolid concentrations in serum or plasma were determined by an HPLC method with ultraviolet (UV) detection. The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of a LC-2010 pump, an LC-2010 autosampler, a LC-2010 UV detector and a LC-2010 column oven. Data were collected and analysed using LC solution. Separation was carried out on an octadecyl silane (ODS) hypersil column (Cadenza 5CD-C18, 150 mm × 4.6 mm, 5 µm; Imtakt Co., Kyoto, Japan). As the mobile phase, a solution of 1% orthophosphoric acid, 30% methanol, and 2 g/l heptane sulfonic acid (1:30:69) and pH was adjusted to 5 by the addition of 10 M sodium hydroxide. The pump flow rate was of 1.0 mL/min. The column temperature was maintained at 40◦C. The wavelength of optimum UV detection was set at 254 nm. Calibration curves were linear over a concentration range of 0.1 to 50 µg/mL for total and unbound linezolid. The intra/inter-day coefficient of variation (CV) was below 5.0%, and the lower limit of

quantification ((LLOQ) was 0.1 μg/mL for both total and unbound of linezolid concentrations.

Population Pharmacokinetics and pharmacodynamics of linezolid

Population pharmacokinetics and pharmacodynamics analysis performed the PPP & D method [43, 44] using the nonlinear mixed effect modeling software NONMEM® version 7.3.0 (ICON Development Solutions, Maryland, USA) with the first-order conditional estimation method with interaction (FOCE-I). The entire procedure of executing model runs, bootstrapping, visual predictive check (VPC) and results management was performed in Wings for NONMEM, and graphical analysis was performed by R (version 2.8.0).

Population Pharmacokinetics

The pharmacokinetics of linezolid assumed a two-compartment distribution model with first-order absorption and elimination (ADVAN13, TOL=9). Pharmacokinetic parameters were clearance (CL), volume of the central and peripheral compartment (VC and VP), inter-compartment clearance (Q), absorption half-life (TABS) and absolute bioavailability (F). The absorption rate constant (Ka) was calculated natural logarithm of 2 divided by TABS.

Between subject variability in pharmacokinetic parameters were modeled with log-normal distribution in the following Equation 1. P_i is the pharmacokinetic parameter for ith individual, P_{POP} is the population mean value of the parameters, and η_i is a normally distributed random variable with mean zero and variance ω^2.

$$P_i = P_{POP} \times e^{\eta_i} \qquad \text{Equation 1}$$

The residual unidentified variability was modeled with combined proportional and additive errors of total and protein unbound concentrations in the following Equation 2. Y_{ij} is the jth measured concentration in the ith subject, Y_{PREDij} is the predicted concentration based

on the model. ε_{CV} and ε_{SD} are the combined proportional and additive error model components, respectively, with mean zero and variance σ^2 for concentration. The fraction of unbound plasma protein binding was estimated from the relationship between total concentrations and unbound concentrations.

$$Y_{ij} = Y_{PREDij} \times (1 + \varepsilon_{CV}) + \varepsilon_{SD} \quad \text{Equation 2}$$

The fraction unbound (fu) was estimated by predicting total plasma concentration from predicted unbound concentrations in plasma. In five samples where the data below the LLOQ of concentrations of linezolid the value was treated as missing.

Covariate model

F_{SIZE} was applied to standardize the pharmacokinetic parameters with an assumption of a standard body weight (TBW) of 70kg (Equation 3) [45, 46]. The allometric exponent (PWR) of F_{SIZE} was fixed to 0.75 for CL and Q, 1 for VC and VP.

$$F_{SIZE} = \left(\frac{TBW}{70}\right)^{PWR} \quad \text{Equation 3}$$

Differences associated with age were described on the basis of the fractional change in a pharmacokinetic parameter. F_{AGE} of CL was defined (Equation 4) and centered around the patient's median age (year). K_{AGECL} is the age parameter for CL. F_{AGE} was fixed to 1 for the model of Q, VC and VP.

$$F_{AGECL} = e^{(K_{AGECL} \times (AGE - AGE_{median}))} \quad \text{Equation 4}$$

Creatinine clearance (CLcr) was calculated by the Cockcroft-Gault formula [47] standardised to a total body weight of 70 kg. Renal function (RF) was normalised to standard CLcr ($CLcr_{STD}$) of 6 L/h/70 kg (100 mL/min/70 kg) by Equation 5 [48, 49].

$$RF = \frac{CLcr}{CLcr_{STD}} \quad \text{Equation 5}$$

There were 4 younger patients (age 1, 5, 8 and 13 y). RF was assumed to be 0.5 in these sick hospitalized patients. RF was included in the model using a linear independent

combination of renal and non-renal clearance parameters by Equation 6. $CL_{OVERALL}$ is the overall population value of parameter. $CL_{non\text{-}renal}$ was non-renal clearance and CL_{renal} is renal clearance.

$$CL_{OVERALL} = (CL_{non-renal} \times F_{AGECL} + CL_{renal} \times RF) \quad \text{Equation 6}$$

The covariate factors was combined to predict linezolid clearance (CL_{GRP}). Group clearance includes covariates used to characterize that specific group's pharmacokinetic parameters (Equation 7).

$$CL_{GRP} = CL_{OVERALL} \times F_{SIZE} \quad \text{Equation 7}$$

Pharmacokinetic parameter estimates are for the disposition of unbound linezolid concentration.

Mixture model

The decrease of platelets by linezolid exposure was assumed to occur with one of two mechanisms in each patient. These mechanisms are inhibition of formation of platelets (PDI) or stimulation of the elimination (PDS) of platelets (**Error! Reference source not found.**).

A mixture model was used to identify the fraction (F) of patients in the study population who were best described by a linezolid inhibitory effect on platelet formation (Equation 8).

$$\text{Inhibit synthesis of platelets ; } F_{POP\_inhibit} \left(0 \leq F_{POP\_inhibit} \leq 1\right) \quad \text{Equation 8}$$

$$\text{Stimulate the elimination of platelets ; } F_{POP\_stimulate} = 1 - F_{POP\_inhibit}$$

Population pharmacokinetic pharmacodynamic modeling

The time course of linezolid induced reduction of platelets count is based on a semi-mechanistic model for myelosuppression [38]. The model is composed of a compartment representing pro-genitor cells in the bone marrow, a compartment of systemic

circulating platelets, and a link between them through three transit compartments reflecting platelet maturation (Equation 9). The inter-compartment transit rate constant (Ktr) was estimated from the mean transit time (MTT) so that Ktr=(1+Ntr)/MTT where Ntr is the number of transit compartments. Circulating platelets (PLTCIRC) were eliminated by a first-order process with half-life (PLTHALF). PLTHALF was estimated and the corresponding rate constant Kcirc was calculated from the natural logarithm of 2 divided by PLTHALF. The initial platelet count in the PLTFORM, Transit 1, Transit 2 and Transit 3 compartments was calculated from PLTCIRC0 x Kcirc/Ktr and in the PLTCIRC compartment it was set to PLTZERO, where PLTZERO is the platelet count in blood before starting linezolid.

$$\frac{d\text{PLTFORM}}{dt} = RFORM \times \text{FBACK} \times \text{PDI} - \text{Ktr} \times \text{PLTFORM} \qquad \text{Equation 9}$$

$$\frac{d\text{Transit 1}}{dt} = \text{K}_{\text{tr}} \times \text{PLTFORM} - \text{K}_{\text{tr}} \times \text{Transit 1}$$

$$\frac{d\text{Transit 2}}{dt} = \text{Ktr} \times \text{Transit 1} - \text{Ktr} \times \text{Transit 2}$$

$$\frac{d\text{Transit 3}}{dt} = \text{Ktr} \times \text{Transit 2} - \text{Ktr} \times \text{Transit 3}$$

$$\frac{d\text{PLTCIRC}}{dt} = \text{Ktr} \times \text{Transit 3} - \text{Kcirc} \times \text{PLTCIRC} \times \text{PDS}$$

The rate of formation of platelets (RFORM) in the platelet formation compartment (PTFORM) was assumed to be driven either by proliferation of cells in the formation compartment or from a constant stem cell precursor (Equation 10).

$$\text{RFORM} = \text{Ktr} \times \text{PLTFORM} \text{ ; proliferation} \qquad \text{Equation 10}$$

$$\text{RFORM} = \text{Ktr} \times \text{PLTZERO} \text{ ; stem cell}$$

An empirical feedback model (FBACK) was used to describe the effect of endogenous growth factors with change the formation rate when the platelet count changes relative to the baseline platelet count (PLTZERO) (Equation 11).

$$\mathrm{FBACK} = \left(\frac{\mathrm{PLTCIRC}}{\mathrm{PLTZERO}}\right)^{-\gamma} \quad \text{Equation 11}$$

The effect of linezolid was implemented either by an inhibitory (PDI) effect on formation of platelets (RFORM) or a stimulatory (PDS) effect on platelet elimination (Kcirc) (Equation 12).

$\mathrm{PDI} = 1 - \mathrm{Edrug}$; Inhibition Equation 12

$\mathrm{PDS} = 1 + \mathrm{Edrug}$; Stimulation

The pharmacodynamic model for linezolid (Edrug) was either a linear or an Emax model (Equation 13).

$\mathrm{Edrug} = \mathrm{SLOPE} \times \mathrm{C_{TOTAL}}$; linear Equation 13

$\mathrm{Edrug} = \frac{\mathrm{Emax} \times \mathrm{C_{TOTAL}}}{\mathrm{C50} + \mathrm{C_{TOTAL}}}$; Emax

The model was implemented as a system of differential equations. All compartments were initialized to PLTZERO.

Model evaluation and validation

To test the significance of various factors that influenced the pharmacokinetic - pharmacodynamics parameters, the value of the objective function (OFV) determined in the NONMEM® fitting routine was used. The difference in OFV (ΔOFV) obtained by comparing each model was asymptotically distributed according to the chi-squared test with the degree of freedom being equal to the difference in the number of parameters between the two models. The significance level was set to p <0.05 (ΔOFV: 3.84).

Non-parametric bootstrap was used to estimate uncertainty [50]. The final model was fit repeatedly to 100 additional bootstrap datasets. The average, standard deviation (SD), relative standard error (%RSE), and 95% confidence intervals (CIs) were calculated from the empirical bootstrap distribution and compared estimates from the original dataset. A prediction-corrected VPC (pcVPC) was used to check the distribution of observed and

predicted percentiles [51]. The VPC was evaluated by comparing the observed concentrations with 90% percentile intervals (PIs) and 95% CIs simulated from the final parameters.

RESULTS

Population Pharmacokinetics of Linezolid

A total of 493 blood linezolid total concentrations and 380 unbound linezolid concentrations from 81 patients were available to develop the population pharmacokinetic model. There was no improvement in the fit (ΔOFV -3.73, df=2, P=0.15) with a previously described model [52] involving a time related effect of linezolid exposure to reduce elimination clearance. The final model contained nine estimated pharmacokinetic parameters.

Plasma protein binding was linearly related to linezolid unbound concentration. There was no improvement in the fit when a saturable binding model was used (ΔOFV -0.522, df=2, P=0.77). The population estimated protein binding percentage was 18%. There was no detectable population parameter variability for the unbound fraction. The pharmacokinetic parameter estimates from the original data and the bootstrap distribution are presented in Error! Reference source not found..

The pharmacokinetic model parameters are shown in Equation 14.

$$\mathrm{CL\ (L/h)} = \left(1.86 \times e^{-0.0205 \times (\mathrm{AGE}-69)} + 1.44 \times \mathrm{RF}\right) \times \left(\frac{\mathrm{TBW}}{70}\right)^{0.75} \qquad \text{Equation 14}$$

$$\mathrm{VC\ (L)} = 22.9 \times \left(\frac{\mathrm{TBW}}{70}\right)$$

$$\mathrm{VP\ (L)} = 24.7 \times \left(\frac{\mathrm{TBW}}{70}\right)$$

$$\mathrm{Q\ (L/h)} = 10.9 \times \left(\frac{\mathrm{TBW}}{70}\right)^{0.75}$$

The pharmacokinetic model described the observed data well. The model validation using pcVPC also confirmed an acceptable agreement between the observed data and

model-based simulated values (**Error! Reference source not found.** and **Error! Reference source not found.**). The median of the observed values was within the 95% confidence interval of the predictions but tended to be higher than the median prediction.

Mixture model

A total of 575 platelet counts from 80 patients were available to develop the turnover and pharmacodynamic model. The estimated mixture model fraction of patients with platelet count decreased due to inhibition of formation was 0.97 ($F_{POPinhib}$) thus the fraction due to stimulation of loss ($F_{POPstim}$) was 0.03 Based on assignment of patients to each mechanism 78 patients had platelet formation inhibited with linezolid and 2 patients had platelet loss stimulated with linezolid treatment.

Population pharmacokinetic pharmacodynamic modeling

A model with 3 transit compartments adequately described the time course of thrombocytopenia. A more complex model with 30 compartments for platelet formation and elimination [37] did not improve the fit.

A linear pharmacodynamic model was chosen to describe PDI and an Emax model for PDS. Renal function (RF) was investigated to see if it affected the linezolid slope of PDI. RF had no significant effect on slope (ΔOFV -0.704, df=1, P=0.4). Modeling platelet turnover using proliferation of cells in the formation compartment significantly improved the fit when compared with a constant stem cell precursor (ΔOFV -80.772, df=0). The fit was not worsened by assuming Kcirc was the same as Ktr so we assume Kcirc=Ktr. Removing the feedback component of the model worsened the fit considerably (ΔOFV 169.8, df=1, P<1e-38).

The final model contained eight estimated parameters including a mixture parameter. The results of pharmacodynamic parameter estimates of the final model and bootstrap

parameter average and 95% empirical bootstrap percentiles from 100 bootstraps are presented in Error! Reference source not found.. The RSE were small (< 30%) for most pharmacokinetic-pharmacodynamic (PKPD) and turnover parameters. Even though the fit was better with an Emax model than a linear model (ΔOFV: 21.080, df=2, P=2.6e-5) the bootstrap RSE of SMAX and SC50 were large (43%, 339% respectively) indicating that the SC50 estimate was particularly uncertain. The model evaluation using pcVPC confirmed an acceptable agreement between the observed data and model-based simulated values (**Error! Reference source not found.**). The median of the observed values was within the 95% confidence interval of the predictions.

A simulation was performed model to demonstrate linezolid-induced thrombocytopenia in patients using parameter estimates from the combined PKPD and turnover model. Simulations of predicted platelet count of PDI and PDS models were performed with linezolid 600 mg every 12 h for at least two weeks as shown in Error! Reference source not found..

When inhibition of platelet formation is assumed then the predicted nadir of platelet count is at 14 days after linezolid administration. On the other hand, when stimulation of loss is assumed then the platelet count drops sharply to reach the predicted nadir after 2 days.

The platelet count and linezolid concentration profiles for representative patients having different dosage and linezolid administration periods are also shown in **Error! Reference source not found.**. Three representative patients with PDI (ID 1, 58 and 64) and the 2 patients with PDS (ID 37 and 55) are shown. The profiles of individual predicted value and observed value for both PDI model and PDS model are close to each other.

DISCUSSION

The mechanism of linezolid induced thrombocytopenia had not yet been elucidated. This study has tried to identify patterns of platelet count associated with two fundamental types of mechanism for thrombocytopenia. A population pharmacokinetic-pharmacodynamic modeling approach was applied to predict linezolid associated effects on platelet turnover either due to myelosuppression or enhanced platelet destruction after oral and/or intravenous infusion of linezolid administered to patients with MRSA infections. By identifying different time courses of thrombocytopenia we hope to help clinicians recognize linezolid induced thrombocytopenia and understand how to manage linezolid dosing in patients with MRSA.

The population pharmacokinetics of linezolid have been previously described using non-compartmental and compartmental models [20, 24, 34, 37, 52-60]. The pharmacokinetic analysis of the present study was performed using a two-compartment model with first-order absorption and first-order elimination.

Both the unbound and total plasma concentrations of linezolid were modelled simultaneously. It is generally accepted that only unbound concentrations are responsible for pharmacological beneficial activity and side effects [61-65]. Most previous reports have been limited to the use of total concentrations [20, 24, 34, 37, 52-60]. The estimated protein binding of linezolid in blood was 18%. There was no detectable between subject variability in fu nor variation of fu with observed concentrations. This result is in agreement with a previous study on linezolid pharmacokinetics [66, 67], and with the findings of Yagi et al. [68].

We have used total body weight and theory based allometry to identify the relationship between body size and pharmacokinetic parameters. Brier et al. reported that the CL of linezolid did not change with renal function [18]. Other reports did not investigate if renal function influenced CL of linezolid [52-60].

Taubert et al. [60] have described that fibrinogen and antithrombin concentrations, lower concentrations of lactate, and the presence of acute respiratory distress syndrome are

significant covariates for CL. However, they were unable to identify that renal function influenced CL of linezolid which may be due to their empirical approach with only 52 critically ill patients. Other reports have clearly identifed that impaired renal function is associated with lower CL [20, 24, 34, 37].

We used a size independent measure of renal function to account for renal impairment and estimate both renal and non-renal components of clearance. This clearly demonstrated the important role of renal elimination of linezolid. After accounting for the effects of size, renal function and plasma protein binding we found there was a small decrease in non-renal clearance with increasing age (2%/y).

A comparison of our estimates of PK parameters with those reported in the literature is shown in Error! Reference source not found.. CL was somewhat lower than reported by others although it is difficult to compare estimates when the original values were not reported in a standardized fashion. This was particularly challenging when renal function was not included in the reported model. Studies including healthy subjects might be expected to have higher CL but there was no evidence for this.

The pc-VPC of linezolid unbound concentrations (Error! Reference source not found.) shows that predictions match observed concentrations initially but from day 3 to day 14 the observed values are under predicted. This would be consistent with the proposal by Plock et al. [52] that linezolid inhibits its own metabolism although the magnitude of the effect observed here is much smaller (10%) than that predicted (75%). Implementation of this model did not improve the fit. It is unlikely that changes in plasma protein binding would cause this effect because linezolid is bound to albumen and albumen is reduced in sick patients [69, 70].

The estimated parameters describing platelet turnover were similar to those of previous studies [34, 37]. The results of our data analysis indicated that the population mean of MTT, γ with absolute value and PLTZERO on the pharmacodynamics were 113 h, 0.187,

206000 /μL, respectively. A comparison of the parameters with those in this study is shown in Error! Reference source not found.. The estimate platelet turnover time (MTT) and feedback parameter γ and linezolid potency (SLOPE) in the current study were similar to that reported by Sasaki et al. [34]. Boak et al. reported a MTT about 50% longer and a γ 5 times larger with a SLOPE 10 times larger [37].

Previous reports regarding linezolid-induce thrombocytopenia proposed two mechanisms involving increased elimination of platelets either by non-immune mediated thrombocytopenia caused by suppressing bone marrow [34, 36, 37, 71], or by linezolid-induced platelet destruction [40, 42]. Loo AS et al. suggested that both of these mechanisms of linezolid-induced thrombocytopenia may involve immune related pathways [72].

The mechanism-based turnover model we have described for linezolid-induced thrombocytopenia involving either inhibition of platelet formation or stimulation of platelet elimination. The platelet turnover models described in previous studies of linezolid explain the decrease of platelets by an inhibitory mechanism without exploring the possibility of stimulated elimination [34, 37]. Sasaki et al. reported linezolid inhibition of platelet proliferation [34]. On the other hand, Boak et al. reported linezolid inhibition of platelet stem cells [37]. We tested both of these platelet inhibition models and found a better fit based on inhibition of platelet proliferation. We are not aware of a specific mechanism explaining how linezolid impairs platelet proliferation.

Based on a mixture model for the distribution of patient responses it appears that linezolid-induced stimulation of platelet elimination occurred in only 3% of patients compared with 97% for linezolid-induced inhibition of platelet formation. It is not possible to directly distinguish if the stimulation of elimination mechanism is immune mediated or no [72, 73]. However, the low value for SC50 (0.33 mg/L total plasma concentration compared to observed concentrations greater than 1 mg/L) is consistent with an immune related

mechanism initiated by very low levels of exposure. Caution is required in this interpretation because of the large RSE and wide bootstrap confidence interval (0.00004 to 1.405 mg/L) for SC50.

Impaired renal function and use of linezolid is associated with a decrease of platelet count [19, 21, 23, 25, 26, 34]. This could be due to an effect of renal impairment on platelets as well as an increased inhibition of platelet proliferation associated with increased linezolid concentrations. Or it could be just due to increased inhibition of platelet proliferation because of increased linezolid concentrations. We could detect no additional effect of renal function (RF) on linezolid induced inhibition. This indicates that linezolid induced thrombocytopenia is due only to linezolid and it is not made worse by renal impairment independently of linezolid concentration.

When the mechanism appears to be inhibition of proliferation the onset of platelet count decrease is delayed and reaches a nadir around 2 weeks (**Error! Reference source not found.**). In contrast, when the mechanism appears to be stimulation of elimination the nadir is reached around 2 days (**Error! Reference source not found.**).

We used our model to predict the time course of linezolid concentration and platelets before and after a period of linezolid treatment in some individual patients to illustrate the typical behavior according to the 2 mechanisms we describe for thrombocytopenia (**Error! Reference source not found.**). We also show the predictions for one patient who appeared to be in the stimulation of elimination group but this was falsely identified because the patient already had thrombocytopenia before starting treatment with linezolid. We removed this patient from the final analysis dataset.

These results are in good agreement with the timing of platelet count nadir described as non-immune mediated thrombocytopenia (inhibition of proliferation) [34, 36, 37, 71, 72] and immune mediated thrombocytopenia (stimulation of elimination) [40, 42, 72]. In view of

these results, we recommend platelet count monitoring should be considered for all patients immediately before the start of linezolid treatment then on day 2 and 4 and week 1, 2 and 3.

A Bayesian dosing method has been developed using the linezolid model described here. It is part of the web based NextDose tool which is implemented for use in a clinical environment. Instructions for access to NextDose are available at www.nextdose.org.

Several limitations of this study warrant mention. First, bone marrow samples were not available. These would allow the differentiation process from hematopoietic cells to megakaryocytes or megakaryocytic differentiation to be studied. It would then be possible to have a clearer understanding of the mechanism of linezolid-induced thrombocytopenia. Second, we cannot be sure that other diseases and/or other drugs had an effect on platelet count. Third, we have very few patients with rapid loss of platelets. However, 2 patients (ID 37 and 55) appeared to have this mechanism as shown by the (**Error! Reference source not found.**). Finally, because heights of patients were not recorded we could not predict normal fat mass [74] and understand better how body composition influences the size relationship for linezolid pharmacokinetic parameters.

We have described the influence of weight, renal function, age and plasma protein binding on the pharmacokinetics of linezolid. This pharmacokinetic model has allowed us to determine that the most common mechanism of thrombocytopenia associated with linezolid is inhibition of platelet proliferation. Increased exposure with renal impairment is predictable and thrombocytopenia avoidable by dose reduction. Target concentration intervention to optimize linezolid exposure is expected to reduce the risk of thrombocytopenia.

REFERENCES

1. Zurenko GE, Yagi BH, Schaadt RD, Allison JW, Kilburn JO, Glickman SE, Hutchinson DK, Barbachyn MR, Brickner SJ. In vitro activities of U-100592 and U-100766, novel oxazolidinone antibacterial agents. Antimicrob Agents Chemother 1996; 40: 839-45.
2. Dryden MS. Linezolid pharmacokinetics and pharmacodynamics in clinical treatment. J Antimicrob Chemother 2011; 66 Suppl 4: iv7-iv15.
3. Lovering AM, Zhang J, Bannister GC, Lankester BJA, Brown JHM, Narendra G, MacGowan AP. Penetration of linezolid into bone, fat, muscle and haematoma of patients undergoing routine hip replacement. J Antimicrob Chemother 2002; 50: 73-77.
4. Tsuji Y, Hashimoto W, Taniguchi S, Hiraki Y, Mizoguchi A, Yukawa E, To H. Pharmacokinetics of linezolid in the mediastinum and pleural space. Int J Infect Dis 2013; 17: E1060-E61.
5. Tsuji Y, Hiraki Y, Matsumoto K, Mizoguchi A, Sadoh S, Kobayashi T, Takemura Y, Sakamoto S, Morita K, Kamimura H, Karube Y. Pharmacokinetics and protein binding of linezolid in cerebrospinal fluid and serum in a case of post-neurosurgical bacterial meningitis. Scand J Infect Dis 2011; 43: 982-85.
6. Tsuji Y, Tashiro M, Ashizawa N, Ota Y, Obi H, Nagura S, Narukawa M, Fukahara K, Yoshimura N, To H, Yamamoto Y. Treatment of Mediastinitis due to Methicillin-resistant Staphylococcus aureus in a Renal Dysfunction Patient Undergoing Adjustments to the Linezolid Dose. Intern Med 2015; 54: 235-39.
7. Noskin GA, Siddiqui F, Stosor V, Hacek D, Peterson LR. In vitro activities of linezolid against important gram-positive bacterial pathogens including vancomycin-resistant enterococci. Antimicrob Agents Chemother 1999; 43: 2059-62.
8. Shinabarger D. Mechanism of action of the oxazolidinone antibacterial agents. Expert Opin Investig Drugs 1999; 8: 1195-202.
9. Cosgrove SE, Qi Y, Kaye KS, Harbarth S, Karchmer AW, Carmeli Y. The impact of methicillin resistance in Staphylococcus aureus bacteremia on patient outcomes: mortality, length of stay, and hospital charges. Infect Control Hosp Epidemiol 2005; 26: 166-74.
10. Shorr AF, Combes A, Kollef MH, Chastre J. Methicillin-resistant Staphylococcus aureus prolongs intensive care unit stay in ventilator-associated pneumonia, despite initially appropriate antibiotic therapy. Crit Care Med 2006; 34: 700-6.
11. Wunderink RG, Niederman MS, Kollef MH, Shorr AF, Kunkel MJ, Baruch A, McGee WT, Reisman A, Chastre J. Linezolid in methicillin-resistant Staphylococcus aureus nosocomial pneumonia: a randomized, controlled study. Clin Infect Dis 2012; 54: 621-9.
12. Rubinstein E, Cammarata S, Oliphant T, Wunderink R, Linezolid Nosocomial Pneumonia Study G. Linezolid (PNU-100766) versus vancomycin in the treatment of hospitalized patients with nosocomial pneumonia: a randomized, double-blind, multicenter study. Clin Infect Dis 2001; 32: 402-12.

13. Wunderink RG, Cammarata SK, Oliphant TH, Kollef MH, Linezolid Nosocomial Pneumonia Study G. Continuation of a randomized, double-blind, multicenter study of linezolid versus vancomycin in the treatment of patients with nosocomial pneumonia. Clin Ther 2003; 25: 980-92.
14. Kollef MH, Rello J, Cammarata SK, Croos-Dabrera RV, Wunderink RG. Clinical cure and survival in Gram-positive ventilator-associated pneumonia: retrospective analysis of two double-blind studies comparing linezolid with vancomycin. Intensive Care Med 2004; 30: 388-94.
15. Kalil AC, Klompas M, Haynatzki G, Rupp ME. Treatment of hospital-acquired pneumonia with linezolid or vancomycin: a systematic review and meta-analysis. BMJ Open 2013; 3: e003912.
16. Walkey AJ, O'Donnell MR, Wiener RS. Linezolid vs glycopeptide antibiotics for the treatment of suspected methicillin-resistant Staphylococcus aureus nosocomial pneumonia: a meta-analysis of randomized controlled trials. Chest 2011; 139: 1148-55.
17. Wang Y, Zou Y, Xie J, Wang T, Zheng X, He H, Dong W, Xing J, Dong Y. Linezolid versus vancomycin for the treatment of suspected methicillin-resistant Staphylococcus aureus nosocomial pneumonia: a systematic review employing meta-analysis. Eur J Clin Pharmacol 2015; 71: 107-15.
18. Brier ME, Stalker DJ, Aronoff GR, Batts DH, Ryan KK, O'Grady M, Hopkins NK, Jungbluth GL. Pharmacokinetics of linezolid in subjects with renal dysfunction. Antimicrob Agents Chemother 2003; 47: 2775-80.
19. Cossu AP, Musu M, Mura P, De Giudici LM, Finco G. Linezolid-induced thrombocytopenia in impaired renal function: is it time for a dose adjustment? A case report and review of literature. Eur J Clin Pharmacol 2014; 70: 23-8.
20. Matsumoto K, Shigemi A, Takeshita A, Watanabe E, Yokoyama Y, Ikawa K, Morikawa N, Takeda Y. Analysis of thrombocytopenic effects and population pharmacokinetics of linezolid: a dosage strategy according to the trough concentration target and renal function in adult patients. Int J Antimicrob Agents 2014; 44: 242-7.
21. Matsumoto K, Takeda Y, Takeshita A, Fukunaga N, Shigemi A, Yaji K, Shimodozono Y, Yamada K, Ikawa K, Morikawa N. Renal function as a predictor of linezolid-induced thrombocytopenia. Int J Antimicrob Agents 2009; 33: 98-99.
22. Pea F, Viale P, Cojutti P, Del Pin B, Zamparini E, Furlanut M. Therapeutic drug monitoring may improve safety outcomes of long-term treatment with linezolid in adult patients. J Antimicrob Chemother 2012; 67: 2034-42.
23. Tsuji Y, Hiraki Y, Matsumoto K, Mizoguchi A, Kobayashi T, Sadoh S, Morita K, Kamimura H, Karube Y. Thrombocytopenia and anemia caused by a persistent high linezolid concentration in patients with renal dysfunction. J Infect Chemother 2011; 17: 70-5.
24. Tsuji Y, Yukawa E, Hiraki Y, Matsumoto K, Mizoguchi A, Morita K, Kamimura H, Karube Y, To H. Population pharmacokinetic analysis of linezolid in low body weight patients with renal dysfunction. J Clin Pharmacol 2013; 53: 967-73.

25. Wu VC, Wang YT, Wang CY, Tsai IJ, Wu KD, Hwang JJ, Hsueh PR. High frequency of linezolid-associated thrombocytopenia and anemia among patients with end-stage renal disease. Clin Infect Dis 2006; 42: 66-72.

26. Nukui Y, Hatakeyama S, Okamoto K, Yamamoto T, Hisaka A, Suzuki H, Yata N, Yotsuyanagi H, Moriya K. High plasma linezolid concentration and impaired renal function affect development of linezolid-induced thrombocytopenia. J Antimicrob Chemother 2013; 68: 2128-33.

27. Pea F, Cojutti P, Dose L, Baraldo M. A one-year retrospective audit of quality indicators of clinical pharmacological advice for personalized linezolid dosing: one stone for two birds? Br J Clin Pharmacol 2015.

28. Birmingham MC, Rayner CR, Meagher AK, Flavin SM, Batts DH, Schentag JJ. Linezolid for the treatment of multidrug-resistant, gram-positive infections: experience from a compassionate-use program. Clin Infect Dis 2003; 36: 159-68.

29. Sotgiu G, Centis R, D'Ambrosio L, Alffenaar JW, Anger HA, Caminero JA, Castiglia P, De Lorenzo S, Ferrara G, Koh WJ, Schecter GF, Shim TS, Singla R, Skrahina A, Spanevello A, Udwadia ZF, Villar M, Zampogna E, Zellweger JP, Zumla A, Migliori GB. Efficacy, safety and tolerability of linezolid containing regimens in treating MDR-TB and XDR-TB: systematic review and meta-analysis. Eur Respir J 2012; 40: 1430-42.

30. Gerson SL, Kaplan SL, Bruss JB, Le V, Arellano FM, Hafkin B, Kuter DJ. Hematologic effects of linezolid: summary of clinical experience. Antimicrob Agents Chemother 2002; 46: 2723-6.

31. Niwa T, Suzuki A, Sakakibara S, Kasahara S, Yasuda M, Fukao A, Matsuura K, Goto C, Murakami N, Itoh Y. Retrospective cohort chart review study of factors associated with the development of thrombocytopenia in adult Japanese patients who received intravenous linezolid therapy. Clin Ther 2009; 31: 2126-33.

32. Attassi K, Hershberger E, Alam R, Zervos MJ. Thrombocytopenia associated with linezolid therapy. Clin Infect Dis 2002; 34: 695-8.

33. Takahashi Y, Takesue Y, Nakajima K, Ichiki K, Tsuchida T, Tatsumi S, Ishihara M, Ikeuchi H, Uchino M. Risk factors associated with the development of thrombocytopenia in patients who received linezolid therapy. J Infect Chemother 2011; 17: 382-7.

34. Sasaki T, Takane H, Ogawa K, Isagawa S, Hirota T, Higuchi S, Horii T, Otsubo K, Ieiri I. Population pharmacokinetic and pharmacodynamic analysis of linezolid and a hematologic side effect, thrombocytopenia, in Japanese patients. Antimicrob Agents Chemother 2011; 55: 1867-73.

35. Hiraki Y, Tsuji Y, Hiraike M, Misumi N, Matsumoto K, Morita K, Kamimura H, Karube Y. Correlation between serum linezolid concentration and the development of thrombocytopenia. Scand J Infect Dis 2012; 44: 60-4.

36. Green SL, Maddox JC, Huttenbach ED. Linezolid and reversible myelosuppression. JAMA 2001; 285: 1291.

37. Boak LM, Rayner CR, Grayson ML, Paterson DL, Spelman D, Khumra S, Capitano B, Forrest A, Li J, Nation RL, Bulitta JB. Clinical population pharmacokinetics and toxicodynamics of linezolid. Antimicrob Agents Chemother 2014; 58: 2334-43.

38. Friberg LE, Henningsson A, Maas H, Nguyen L, Karlsson MO. Model of chemotherapy-induced myelosuppression with parameter consistency across drugs. J Clin Oncol 2002; 20: 4713-21.

39. Harker LA, Marzec UM, Hunt P, Kelly AB, Tomer A, Cheung E, Hanson SR, Stead RB. Dose-response effects of pegylated human megakaryocyte growth and development factor on platelet production and function in nonhuman primates. Blood 1996; 88: 511-21.

40. Bernstein WB, Trotta RF, Rector JT, Tjaden JA, Barile AJ. Mechanisms for linezolid-induced anemia and thrombocytopenia. Ann Pharmacother 2003; 37: 517-20.

41. De Vriese AS, Coster RV, Smet J, Seneca S, Lovering A, Van Haute LL, Vanopdenbosch LJ, Martin JJ, Groote CC, Vandecasteele S, Boelaert JR. Linezolid-induced inhibition of mitochondrial protein synthesis. Clin Infect Dis 2006; 42: 1111-7.

42. Pascoalinho D, Vilas MJ, Coelho L, Moreira P. Linezolid-related immune-mediated severe thrombocytopenia. Int J Antimicrob Agents 2011; 37: 88-9.

43. Zhang L, Beal SL, Sheiner LB. Simultaneous vs. sequential analysis for population PK/PD data I: best-case performance. J Pharmacokinet Pharmacodyn 2003; 30: 387-404.

44. Zhang L, Beal SL, Sheinerz LB. Simultaneous vs. sequential analysis for population PK/PD data II: robustness of methods. J Pharmacokinet Pharmacodyn 2003; 30: 405-16.

45. Holford N, Heo YA, Anderson B. A pharmacokinetic standard for babies and adults. J Pharm Sci 2013; 102: 2941-52.

46. Anderson BJ, Holford NHG. Mechanistic Basis of Using Body Size and Maturation to Predict Clearance in Humans. Drug Metab Pharmacokinet 2009; 24: 25-36.

47. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16: 31-41.

48. Matthews I, Kirkpatrick C, Holford N. Quantitative justification for target concentration intervention--parameter variability and predictive performance using population pharmacokinetic models for aminoglycosides. Br J Clin Pharmacol 2004; 58: 8-19.

49. Mould DR, Holford NH, Schellens JH, Beijnen JH, Hutson PR, Rosing H, ten Bokkel Huinink WW, Rowinsky EK, Schiller JH, Russo M, Ross G. Population pharmacokinetic and adverse event analysis of topotecan in patients with solid tumors. Clin Pharmacol Ther 2002; 71: 334-48.

50. Parke J, Holford NH, Charles BG. A procedure for generating bootstrap samples for the validation of nonlinear mixed-effects population models. Comput Methods Programs Biomed 1999; 59: 19-29.

51. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. AAPS J 2011; 13: 143-51.

52. Plock N, Buerger C, Joukhadar C, Kljucar S, Kloft C. Does linezolid inhibit its own metabolism? - Population pharmacokinetics as a tool to explain the observed nonlinearity in both healthy volunteers and septic patients. Drug Metabolism and Disposition 2007; 35: 1816-23.
53. Abe S, Chiba K, Cirincione B, Grasela TH, Ito K, Suwa T. Population pharmacokinetic analysis of linezolid in patients with infectious disease: application to lower body weight and elderly patients. J Clin Pharmacol 2009; 49: 1071-8.
54. Adembri C, Fallani S, Cassetta MI, Arrigucci S, Ottaviano A, Pecile P, Mazzei T, De Gaudio R, Novelli A. Linezolid pharmacokinetic/pharmacodynamic profile in critically ill septic patients: intermittent versus continuous infusion. Int J Antimicrob Agents 2008; 31: 122-9.
55. Beringer P, Nguyen M, Hoem N, Louie S, Gill M, Gurevitch M, Wong-Beringer A. Absolute bioavailability and pharmacokinetics of linezolid in hospitalized patients given enteral feedings. Antimicrob Agents Chemother 2005; 49: 3676-81.
56. Keel RA, Schaeftlein A, Kloft C, Pope JS, Knauft RF, Muhlebach M, Nicolau DP, Kuti JL. Pharmacokinetics of intravenous and oral linezolid in adults with cystic fibrosis. Antimicrob Agents Chemother 2011; 55: 3393-8.
57. Meagher AK, Forrest A, Rayner CR, Birmingham MC, Schentag JJ. Population pharmacokinetics of linezolid in patients treated in a compassionate-use program. Antimicrob Agents Chemother 2003; 47: 548-53.
58. Welshman IR, Sisson TA, Jungbluth GL, Stalker DJ, Hopkins NK. Linezolid absolute bioavailability and the effect of food on oral bioavailability. Biopharm Drug Dispos 2001; 22: 91-7.
59. Whitehouse T, Cepeda JA, Shulman R, Aarons L, Nalda-Molina R, Tobin C, MacGowan A, Shaw S, Kibbler C, Singer M, Wilson AP. Pharmacokinetic studies of linezolid and teicoplanin in the critically ill. J Antimicrob Chemother 2005; 55: 333-40.
60. Taubert M, Zoller M, Maier B, Frechen S, Scharf C, Holdt LM, Frey L, Vogeser M, Fuhr U, Zander J. Predictors of Inadequate Linezolid Concentrations after Standard Dosing in Critically Ill Patients. Antimicrob Agents Chemother 2016; 60: 5254-61.
61. Bailey EM, Rybak MJ, Kaatz GW. Comparative effect of protein binding on the killing activities of teicoplanin and vancomycin. Antimicrob Agents Chemother 1991; 35: 1089-92.
62. Nix DE, Matthias KR, Ferguson EC. Effect of ertapenem protein binding on killing of bacteria. Antimicrob Agents Chemother 2004; 48: 3419-24.
63. Schmidt S, Rock K, Sahre M, Burkhardt O, Brunner M, Lobmeyer MT, Derendorf H. Effect of protein binding on the pharmacological activity of highly bound antibiotics. Antimicrob Agents Chemother 2008; 52: 3994-4000.
64. Wise R. The clinical relevance of protein binding and tissue concentrations in antimicrobial therapy. Clin Pharmacokinet 1986; 11: 470-82.
65. Zeitlinger MA, Sauermann R, Traunmuller F, Georgopoulos A, Muller M, Joukhadar C. Impact of plasma protein binding on antimicrobial activity using time-killing curves. J Antimicrob Chemother 2004; 54: 876-80.

66. Buerger C, Plock N, Dehghanyar P, Joukhadar C, Kloft C. Pharmacokinetics of unbound linezolid in plasma and tissue interstitium of critically ill patients after multiple dosing using microdialysis. Antimicrob Agents Chemother 2006; 50: 2455-63.

67. Traunmuller F, Schintler MV, Spendel S, Popovic M, Mauric O, Scharnagl E, Joukhadar C. Linezolid concentrations in infected soft tissue and bone following repetitive doses in diabetic patients with bacterial foot infections. Int J Antimicrob Agents 2010; 36: 84-86.

68. Yagi T, Naito T, Doi M, Nagura O, Yamada T, Maekawa M, Sato S, Kawakami J. Plasma exposure of free linezolid and its ratio to minimum inhibitory concentration varies in critically ill patients. Int J Antimicrob Agents 2013; 42: 329-34.

69. Don BR, Kaysen G. Serum albumin: relationship to inflammation and nutrition. Semin Dial 2004; 17: 432-7.

70. Sleep D. Albumin and its application in drug delivery. Expert Opin Drug Deliv 2015; 12: 793-812.

71. Ebeling F, Helminen P, Anttila VJ. Appearance of ring sideroblasts in bone marrow during linezolid therapy. Scand J Infect Dis 2009; 41: 480-2.

72. Loo AS, Gerzenshtein L, Ison MG. Antimicrobial drug-induced thrombocytopenia: a review of the literature. Semin Thromb Hemost 2012; 38: 818-29.

73. Aster RH, Bougie DW. Drug-induced immune thrombocytopenia. N Engl J Med 2007; 357: 580-7.

74. Anderson BJ, Holford NHG. Mechanistic basis of using body size and maturation to predict clearance in humans. Drug Metab Pharmacokinet 2009; 24: 25-36.

Table 1

Demographic and clinical data for the study population of patients receiving linezolid.

			Observation Interval	
	Number	Median	Lower 2.5%	Upper 97.5%
Total patients	81			
Male	51			
Female	30			
Administration route				
i.v	54			
p.o.	13			
both	14			
Observed total concentration (mg/L)	493	11.2	2.0	50.7
Observed unbound concentration (mg/L)	380	1.9	0.3	8.8
Protein binding ratio (%)		17.0	7.6	33.3
Observed platelet count (10^3/µL)	575	160	22	463
Observed hemoglobin level (g/dL)	595	8.6	6.1	15.0
Age (year)		69	8	85
Total body weight (kg)		53.2	21.0	99.5
Diagnosis infections				
Sepsis	26			
Wound, Skin and soft tissue	25			
Pneumonia	14			
Abscess	8			
Osteomyelitis	6			
Undetermined	2			
AST(IU/L)		22	10	163

ALT (IU/L)	20	4	190
Serum creatinine (mg/dL)	0.80	0.20	7.49
CLcr (mL/min)	59.6	5.6	188.4

PI, percentile interval; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CLcr, creatinine clearance.

Table 2

Comparison of population pharmacokinetic-pharmacodynamic parameters estimates for final model with estimates from 100 bootstrap sample

Parameter	Description	Units	Final model estimate	Bootstrap sample estimates			
				Average	95% PI		RSE%
					Lower 2.5%	Upper 97.5%	
Population mean							
Pharmacokinetics							
$CL_{non\text{-}renal}$	Non-renal clearance	L/h	1.86	1.76	1.29	2.17	14%
CL_{renal}	Renal clearance	L/h	1.44	1.42	0.83	2.20	25%
VC	Volume of the central compartment	L	22.9	19.3	8.1	29.4	29%
VP	Volume of the peripheral compartment	L	24.7	24.4	16.7	34.2	18%
Q	Inter-compartment clearance	L/h	10.9	10.5	2.3	23.5	90%
TABS	Absorption half-life	h	3.61	5.07	2.13	19.26	70%
F	Absolute bioavailability		0.922	0.895	0.747	0.999	8%
K_{AGECL}	Age parameter for CL		-0.021	-0.021	-0.030	-0.009	-25%
FU	Fraction of unbound protein binding		0.823	0.823	0.809	0.836	1%
Pharmacodynamics							

$F_{inhibit}$	Fraction of patients with inhibit synthesis of platelets		0.969	0.949	0.867	1	3%
MTT	Mean transit time	h	113.0	103.5	65.4	130.0	15%
γ	Feedback parameter		-0.187	-0.164	-0.258	-0.061	-29%
PLTZERO	Baseline platelet count	/μL	206000	204451	174000	234100	8%
SLOPE	Slope of inhibition effect (total plasma concentration)	1/(mg/L)	0.00566	0.00507	0.00248	0.00725	23%
SMAX	Maximal extent of stimulation effect		2.55	2.31	0.03	4.06	43%
SC50	Linezolid total plasma concentration producing 50% of the maximum stimulation effect	mg/L	0.00364	0.324	0.00004	1.405	339%
Between-subject variability (BSV[1])							
CL			0.369	0.366	0.267	0.464	14%
VC			1.421	1.518	1.065	2.348	22%
VP			0.050	0.206	0.024	0.629	78%
Q			1.822	1.624	0.585	2.447	31%
TABS			0 FIXED				
F			0 FIXED				
MTT			0.239	0.205	0.002	0.444	66%
γ			0.307	0.225	0.003	0.521	82%

PLTZERO			0.570	0.567	0.437	0.669	10%
SLOPE			0.473	0.482	0.176	0.759	33%
SMAX			0 FIXED				
SC50			0 FIXED				
Residual unidentified variability (RUV^2)							
RUV_{PROP_TOTAL}	Proportional residual unidentified variability of total concentration		0.318	0.311	0.258	0.356	7%
RUV_{ADD_TOTAL}	Additive residual unidentified variability of total concentration	mg/L	0.251	0.301	0.003	0.806	77%
$RUV_{PROP\_UNBOUND}$	Proportional residual unidentified variability of protein unbound concentration		0.319	0.313	0.256	0.357	8%
$RUV_{ADD\_UNBOUND}$	Additive residual unidentified variability of protein unbound concentration	mg/L	0.034	0.061	0.000	0.729	284%
RUV_{PROP_PLT}	Proportional residual unidentified variability of protein unbound concentration		0.234	0.242	0.204	0.273	8%

1=BSV calculated from sqrt (NONMEM OMEGA); 2=RUV estimated using THETA

PI, percentile interval; RSE, relative standard error;

Table 3

Comparison of pharmacokinetic and parameters (average, BSV%) of linezolid estimated in this study with those in the literature

	Type of subject	CL (L/h/70 kg)	VC (L/70 kg)	VP (L/70 kg)	Q (L/h/70 kg)	Ka (h^{-1})	TABS (h)	F	T half (h)
This study:	Patient	3.3 (36.9)	22.9 (142)	24.7 (5.0)	10.9 (182)	0.19	3.61	0.92	10.0
Literature studies:									
Matsumoto et al. [20]	Patient	4.61 (30.5) #	27.6 #						4.1
Sasaki et al. [34]	Patient	3.87 (35.2)	40.6 (30.8)						7.3
Boak et al. [37]	Patient	6.72 (48.9)	47.7 (3.6)			4.04	0.17 (14.7)		4.7
Abe et al. [53]	Patient	5.34 (46.6)	47.3 (25.9)			0.58 (327)	1.19		6.1
Adembri et al. [54]	Patient	13.0 #	55.7						3.0
Beringer et al. [55]	Patient	8.82 #	60.2			0.75	0.92	0.88	4.7
Keel et al. [56]	Patient	9.54 (36.3) #	26.8 #	17.3 (85.8)	104	1.91	0.36	0.85 (23.0)	3.2
Meagher et al. [57]	Patient	7.38 (50.3)	42.7 (22.7)	28.2	9.09	5.73	0.12		6.7
Plock et al. [52]	Healthy and	2.67 $ (41.7) #	20.0 (40.1)	28.9 (34.8)	75.0	1.81 (72.4)	0.38		3.1

	Patient									
Welshman et al. [58]	Healthy	8.76 #	62.6						1.03	5.0
Whitehouse et al. [59]	Patient	3.41 (48.1) #	44.4 (22.4)	240 (146)	7.48					57.8
Taubert et al. [60]	Patient	7.92 (58.0) #	13.5 (37.0)	26.6		1.72	0.40			3.5

Literature estimate of parameters were standardized based on TBW of 70 kg and CLcr of 6 L/h/70 kg when possible (#=not standardized); Between-subject variability (BSV%) was calculated from 100 x sqrt (NONMEM OMEGA); CL, clearance; VC, volume of the central compartment; VP, volume of the peripheral compartment; Q, inter-compartment clearance; Ka, absorption rate constant; TABS, absorption half-life; F, absolute bioavailability, T half, elimination half-life

Ka was calculated as natural logarithm of 2 (Ln (2)) divided by TABS or TABS as Ln (2)/Ka; T half was calculated as Ln (2)*(VC+VP)/CL. $=calculated at 10 mg/L total concentration.

Table 4

Comparison of pharmacodynamic parameters (average, BSV%) of linezolid estimated in this study with two literature

Authors	Type of subject	MTT (h)	γ	PLTZERO (/μL)	SLOPE 1/(mg/L)	SMAX	SC50 (mg/L)
This study:	Patient	113 (23.9)	-0.187 (30.7)	206000 (57.0)	0.00566 (47.3)	2.55	0.00364
Literature studies:							
Sasaki et al. [34]	Patient	110 (33.9)	-0.203	253000 (45.9)	0.00416 (93.8)		
Boak et al. [37]	Patient	163 (20.3)	-1.02	252000 (65.1)	0.055 (at 10 mg/L)		

Between-subject variability (BSV%) was calculated from 100 x sqrt (NONMEM OMEGA); MTT, mean transit time; γ, feedback parameter; PLTZERO, baseline platelet count; SLOPE, slope of inhibition effect; SMAX, maximal extent of stimulation effect; SC50, linezolid total plasma concentration producing 50% of the maximum stimulation effect.

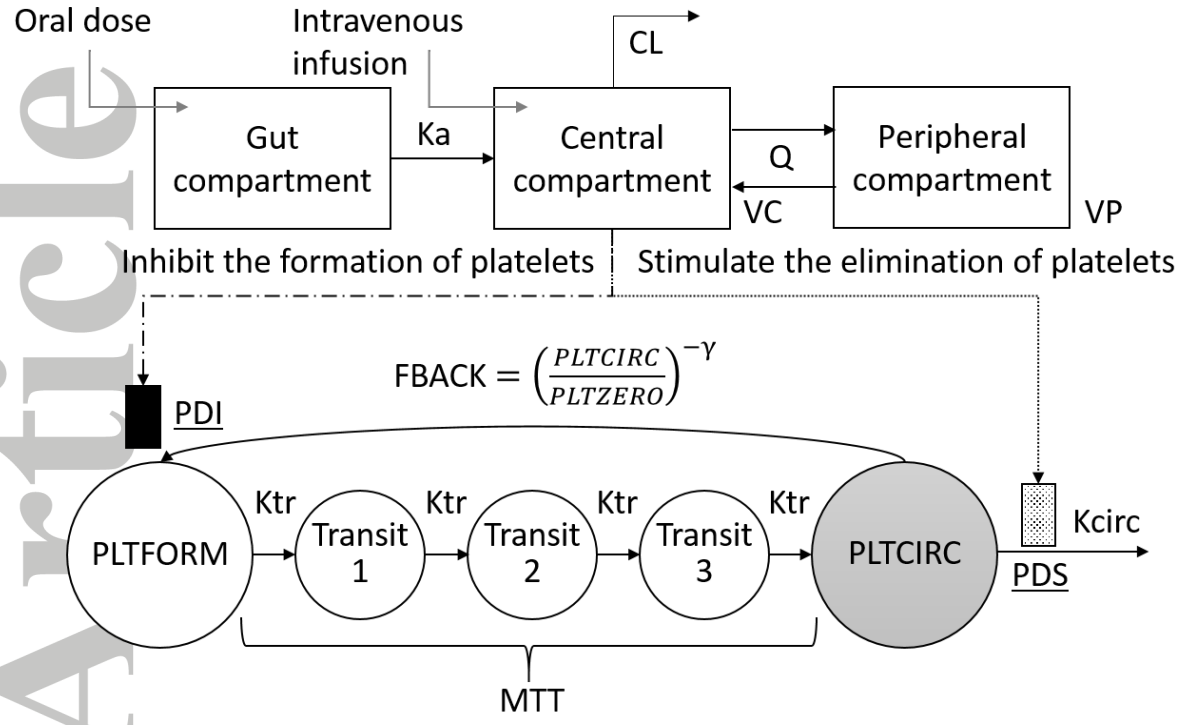

Figure 1

Schematic representation of the structural pharmacokinetic model for linezolid and pharmacodynamic model for platelet time course.

PDI, inhibition effect; PDS, stimulation effect; PLTFORM, initial rate of formation of platelets; Ktr, inter-compartment transit rate constant; MTT, mean transit time; PLTCIRC, circulating platelets; Kcirc, rate constant of PLTCIRC; PLTZERO, baseline platelet count; FBACK, empirical feedback model; CL, clearance; VC and VP, volume of the central and peripheral compartment; Q, inter-compartment clearance; Ka, absorption rate constant. The final model uses Ktr=Kcirc.

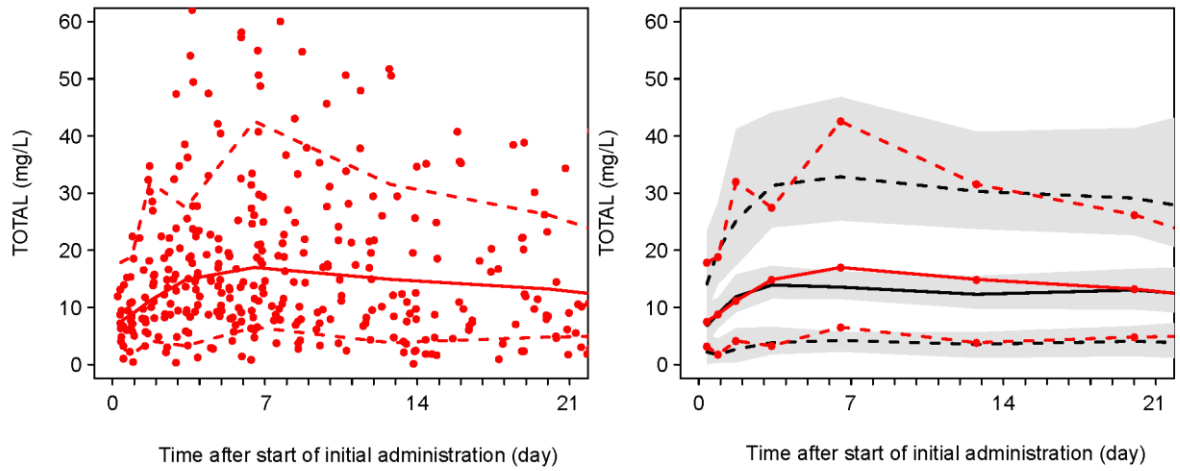

Figure 2

Model qualification using prediction-corrected visual predictive checks (pc-VPC) for total linezolid concentration.

The left hand plots are a scatterplot of the measurements with 5,50 and 95%iles.

pc-VPC showing the 5th, 50th and 95th percentiles for observed and predicted values.

TOTAL, total linezolid concentration; red circles, observed linezolid concentration; red solid line, median observed concentration; red dashed lines, 5th and 95th percentiles the observed linezolid concentrations; black solid line, median predicted linezolid concentration in 100 simulated subsets of total dataset; black dashed lines, 5th to 95th percentiles of the predicted linezolid concentrations; Grey-shaded areas represent 95% confidence intervals of the prediction percentiles

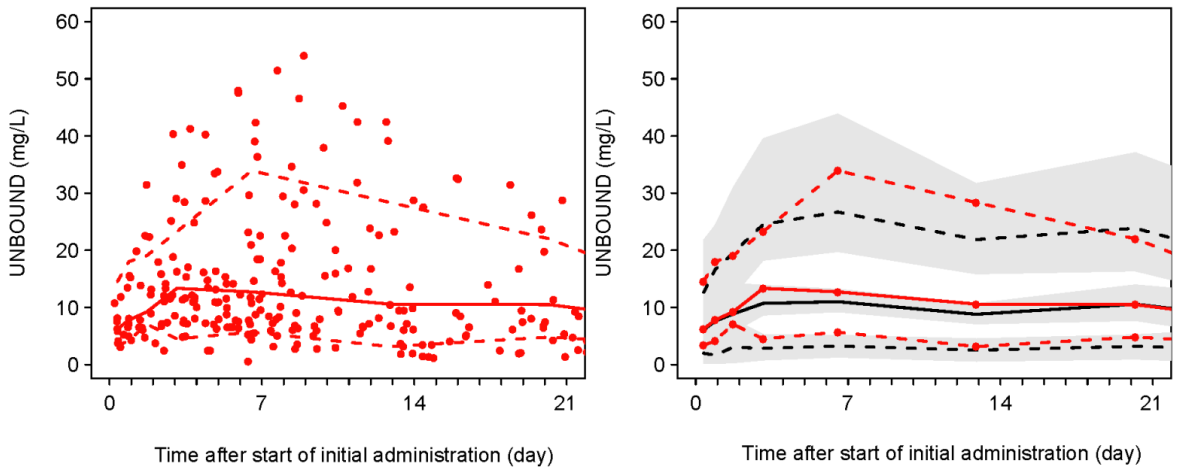

Figure 3

Model qualification using prediction-corrected visual predictive checks (pc-VPC) for unbound plasma linezolid concentration.

The left hand plots are a scatterplot of the measurements with 5,50 and 95%iles.

pc-VPC showing the 5th, 50th and 95th percentiles for observed and predicted values.

UNBOUND, unbound linezolid concentration; red circles, observed linezolid concentration; red solid line, median observed concentration; red dashed lines, 5th and 95th percentiles the observed linezolid concentrations; black solid line, median predicted linezolid concentration in 100 simulated subsets of total dataset; black dashed lines, 5th to 95th percentiles of the predicted linezolid concentrations; Grey-shaded areas represent 95% confidence intervals of the prediction percentiles

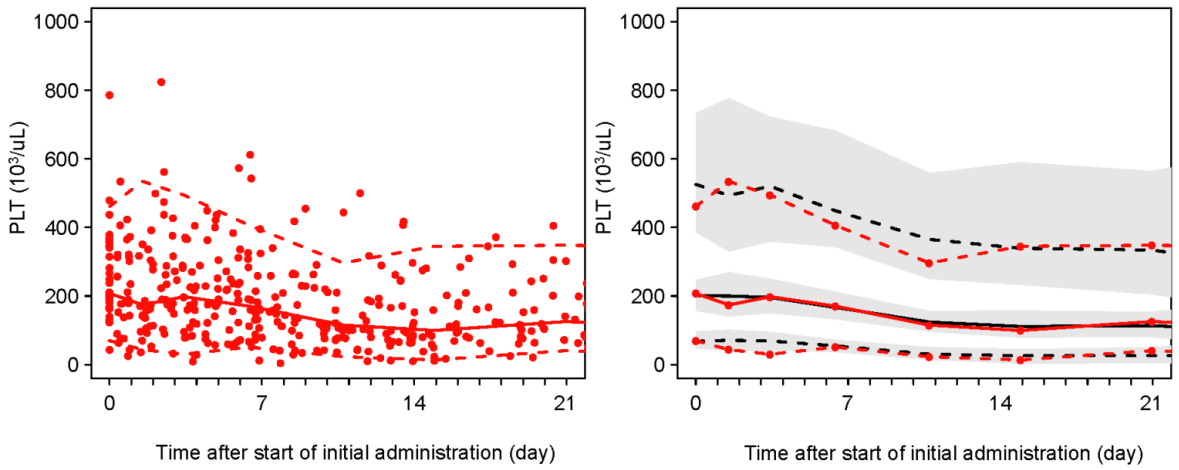

Figure 4

Model qualification using prediction-corrected visual predictive checks (pc-VPC) for platelets count (mixture group 1 - inhibition of proliferation).

The left hand plots are a scatterplot of the measurements with 5,50 and 95%iles.

pc-VPC showing the 5th, 50th and 95th percentiles for observed and predicted values.

PLT, platelets count (mixture group 1); red circles, observed platelets count; red solid line, median observed platelets count; red dashed lines, 5th and 95th percentiles the observed platelets count; black solid line, median predicted platelets count in 100 simulated subsets of total dataset; black dashed lines, 5th to 95th percentiles of the predicted platelets count; Grey-shaded areas represent 95% confidence intervals of the prediction percentiles

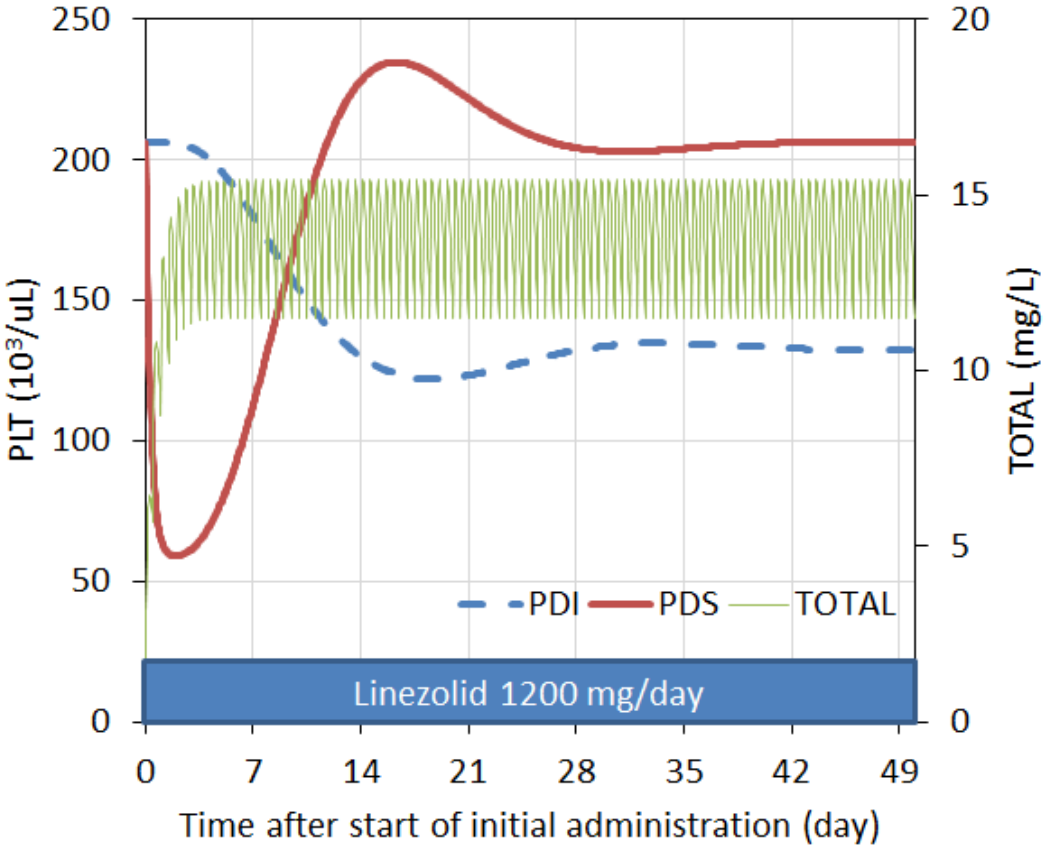

Figure 5

Predicted time course total linezolid plasma concentration and platelet count with inhibition of proliferation (PDS) or stimulation of destruction (PDS) produced by linezolid 1200 mg/day p.o. Simulation using mean parameter based on final model (total body weight of 70 kg, CLcr of 6 L/h/70 kg, age 69, linezolid oral dosage 600 mg q12h)

PDI, platelets count of patient with inhibit synthesis of platelets; PDS, platelets count of patient with stimulate the elimination of platelets; TOTAL, total linezolid concentration;

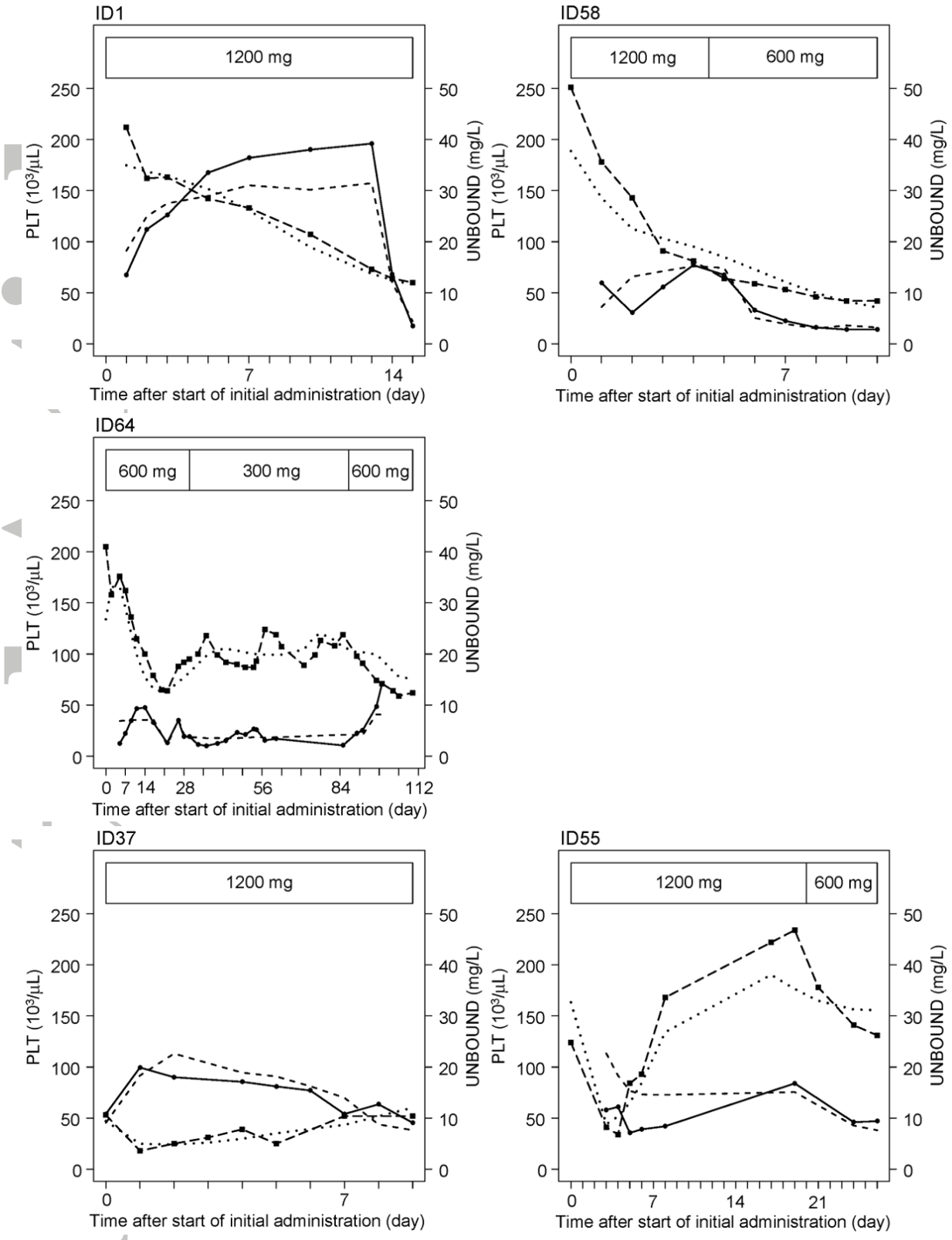

Figure 6

Time course of platelet count and unbound plasma linezolid concentration in representative patients having different dosage and linezolid administration duration.

Patient ID 1, 58 and 64 are patients that are best described by inhibition of synthesis of platelets.

Patient ID 37 and 55 are patients that are best described by stimulation of elimination of platelets.

Boxed areas shows linezolid duration and dosage per day.

PLT, platelets count; UNBOUND, unbound plasma linezolid concentration; closed circle and solid line, observed unbound plasma linezolid concentration; dashed line, predicted unbound plasma linezolid concentration using final model; closed square and long-dashed line, observed platelet count; dotted line, predicted platelet count.