Does linezolid inhibit its own metabolism? – Population pharmacokinetics as a tool to explain the observed nonlinearity in both healthy volunteers and septic patients

N. Plock, C. Buerger, C. Joukhadar, S. Kljucar, C. Kloft

Freie Universitaet Berlin, Institute of Pharmacy, Berlin, Germany (N.P., C.B., C.K.)
University School of Medicine, Vienna, Austria (C.J.)
DRK Kliniken Berlin Westend, Berlin, Germany (S.K.)
Martin-Luther-Universitaet Halle-Wittenberg, Institute of Pharmacy, Halle, Germany (C.K.)
a) Running title: PK of linezolid – does it inhibit its own metabolism?

b) Corresponding author:
Prof. Dr. Charlotte Kloft
Martin-Luther-Universitaet Halle-Wittenberg
Institute of Pharmacy
Dept. Clinical Pharmacy
Wolfgang-Langenbeck-Str. 4
06120 Halle
Phone: +49-(0)345-55 25190
Fax: +49-(0)345-55 27257
Email: charlotte.kloft@pharmazie.uni-halle.de

c) Number of
- text pages: 15
- tables: 1
- figures: 7
- references: 38
- words in abstract: 246
- words in introduction: 663
- words in discussion: 1445

d) Nonstandard abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>KA</td>
<td>absorption rate constant</td>
</tr>
<tr>
<td>CL</td>
<td>clearance</td>
</tr>
<tr>
<td>Q</td>
<td>intercompartmental clearance</td>
</tr>
<tr>
<td>PK</td>
<td>pharmacokinetics</td>
</tr>
<tr>
<td>MAO</td>
<td>monoaminoxidase</td>
</tr>
<tr>
<td>ALAG</td>
<td>lag-time after oral dosing</td>
</tr>
<tr>
<td>RCLF</td>
<td>remaining CL fraction (i.e. non-inhibitable fraction of CL)</td>
</tr>
<tr>
<td>KIC</td>
<td>rate constant into the inhibition compartment</td>
</tr>
<tr>
<td>IC50</td>
<td>concentration in the inhibition compartment yielding 50% of CL inhibition</td>
</tr>
<tr>
<td>V_max</td>
<td>maximum elimination rate</td>
</tr>
<tr>
<td>K_m</td>
<td>Michaelis Menten constant</td>
</tr>
</tbody>
</table>
Abstract

Few studies investigating the population pharmacokinetics of linezolid in critically ill patients have been reported, yielding controversial results. Therefore, a population pharmacokinetic analysis using NONMEM™ was performed in order to thoroughly understand the pharmacokinetics of unbound linezolid in plasma. Data was obtained from 10 healthy volunteers and 24 septic patients. Intensive sampling was performed after single and multiple dosing. The pharmacokinetics of unbound linezolid was best described by a two-compartment model with an absorption rate constant $KA$ (1.81 h⁻¹), a clearance $CL$ (11.1 l/h), volumes of distribution $V_2$ and $V_3$ (20.0 and 28.9 l, respectively) and an intercompartmental clearance $Q$ (75.0 l/h). However, clearance was inhibited over time to 76.4% of its original value, dependent on the concentration in an empirical inhibition compartment. Overall, imprecision of parameter estimates was low to moderate. Comparison of goodness of fit graphics and of the predictive performance revealed that the presented model was superior to previously published models using linear elimination or parallel linear and Michaelis Menten elimination and also to other own investigated model alternatives. The observed nonlinearity in linezolid pharmacokinetics might be a result of an inhibition of the formation of the major linezolid metabolite due to the inhibition of the respiratory chain enzyme activity. This study presents the first attempt to mechanistically explain the observed nonlinearity in linezolid pharmacokinetics. Finally, simulations demonstrated that the model might also serve as a tool to predict concentration-time profiles of linezolid, thus providing a rationale for a more targeted antimicrobial therapy.
Introduction

Linezolid, the first member of the oxazolidinones, is approved for reserve treatment of serious infections such as pneumonia or severe skin and soft tissue infections caused by gram-positive aerobic and anaerobic pathogens such as *staphylococci* and *enterococci* (Pharmacia, 2005). Linezolid acts through inhibition of an early phase of protein synthesis by binding with the 23S rRNA of the ribosomal 50S subunit and by this inhibits the formation of the N-formylmethionyl-tRNA-ribosome-mRNA ternary initiation complex in bacterial translation systems (Swaney et al., 1998). Clinical trials showed that linezolid was generally well tolerated for up to 28 days (French, 2003). Frequent adverse events included headache, nausea, dizziness and vomiting (Chen et al., 2004). In addition, linezolid therapy frequently led to an increase in transaminases (French, 2003). Especially prolonged treatment caused myelosuppression which was reversible after discontinuation of therapy (Green et al., 2001). Moreover, optical neuropathy has been observed after long-term treatment (Meyer et al., 2005).

The pharmacokinetics (PK) of linezolid has been subject to various investigations. Linezolid displayed a plasma protein binding of 31% independent of concentration (Slatter et al., 2001). At steady state, the volume of distribution was 40 to 50 l, which approximates total body water (Diekema and Jones, 2001). Linezolid had a total clearance of 7 l/h and a terminal elimination half-life of approximately 5 h (Perry and Jarvis, 2001). In patients with severely impaired renal function no significant changes in total clearance were observed; thus, a dose adjustment was reported not to be necessary in this patient population (Brier et al., 2003). Of the total amount of linezolid in the body only 30% were eliminated unchanged via the kidneys (Moellerling, 2003) while a major part of the administered linezolid was metabolized by oxidation of its morpholino ring (see figure 1), resulting in two metabolites: an aminoethoxyacetic acid metabolite (metabolite A) and a hydroxyethyl glycine metabolite (metabolite B) that was formed by nonenzymatic oxidation in an *in vitro* setting (Slatter et al.,
2001). In urine, 40% of the dose appeared as metabolite B and 10% as metabolite A while 6% and 1.5% were eliminated via feces as metabolite B and A, respectively (Slatter et al., 2001). The formation of metabolite B was found to be optimal under basic (pH 9.0) conditions which suggests the potential involvement of either an uncharacterized P450 enzyme or an alternative microsomal-mediated oxidative pathway (Wynalda et al., 2000). In addition, its formation was dependent on NADPH (Wynalda et al., 2000).

It has been demonstrated that PK characteristics can differ between healthy volunteers and critically ill patients (Gomez et al., 1999; Brunner et al., 2000; Hanes et al., 2000; Joukhadar et al., 2001; Joukhadar et al., 2002; Tegeder et al., 2002). Nevertheless, pharmacokinetic investigations of linezolid in critically ill patients have been scarce (Meagher et al., 2003; Whitehouse et al., 2005) and, concerning the structural pharmacokinetic model as well as pharmacokinetic parameter estimates, yielded controversial results. Moreover, it has been demonstrated that only the unbound fraction of a drug is pharmacologically active (Kunin et al., 1973; Merrikin et al., 1983; Craig and Ebert, 1989). Therefore, more emphasis should be placed on the investigation of unbound linezolid PK in critically ill, e.g. septic patients. In order to increase the informational value of those studies, informative analysis procedures such as population PK should be adopted. In brief, population PK is a method that is able to analyze concentration-time data of many individuals simultaneously. Moreover, overall variability can be characterized and differentiated in more detail, as e.g. the interindividual variability in drug exposure and the residual variability (Sheiner, 1984).

The primary objective of this investigation was to analyze data derived from a clinical trial that investigated both healthy volunteers and septic patients in order to thoroughly describe and understand the PK of unbound linezolid in plasma after single and multiple dosing. For this purpose, the population pharmacokinetic analysis technique was applied. Moreover, the developed model was to be compared to previously published models and to be evaluated in terms of its predictive performance.
Subjects and Methods

Subjects: A multi-centre, open-labeled, prospective comparative study approved by the local ethics committees in Austria and Germany was conducted to investigate the PK of linezolid in healthy volunteers and septic patients (Buerger et al., 2006; Thallinger C, Buerger C, Plock N, Kljucar S, Kloft C, Joukhadar C. Linezolid concentrations in soft tissues in healthy volunteers, patients presenting sepsis and septic shock, in preparation). In total, 10 healthy volunteers and 24 patients were enrolled in the trial according to previously reported criteria (Buerger et al., 2006). In brief, severe sepsis and septic shock were diagnosed according to the criteria of the ACCP/SCCM Consensus Conference Committee (Bone et al., 1992). Patients were excluded if they were tested positive for HIV or Hepatitis B at the screening visit, received hemodialysis or hemofiltration 3 days prior to or within the first four days of study drug administration, were allergic to linezolid, were concomitantly given MAO inhibitors or during pregnancy and lactation.

Study protocol: In all subjects, the first dose was 600 mg of linezolid (Zyvoxid™, Pharmacia, Erlangen, Germany), administered as a 30-minute intravenous infusion. Patients continued to receive 600 mg linezolid bid as an infusion. In healthy volunteers, all subsequent doses were given orally as a 600 mg linezolid tablet bid. The healthy volunteers had to document the actual time of linezolid intake in a diary. In each subject, blood samples were planned to be collected on two study visits. Visit 1 was conducted beginning with the first linezolid administration. Visit 2 was carried out after multiple dosing, i.e. at least three days after continued linezolid administration.

Sampling and sample storage: The sampling schedule was the same for both study visits. Samples were collected directly before and 20, 40, 60, 80, 100, 120, 140, 160, 180, 210, 240, 270, 300, 330, 360, 390, 420, 450 and 480 min after the start of linezolid administration. Blood was centrifuged at 2550 g for 5 min immediately after sampling and stored at –70°C. Sample transport was carried out using dry ice adhering to a cold chain.
Linezolid quantification: Total and unbound linezolid concentrations in plasma were measured using a previously described HPLC method with an RP-18 stationary phase and UV detection (Buerger et al., 2003). In brief, plasma samples were prepared by using a 50 µl aliquot of each sample. Proteins were precipitated with acetonitrile whereas ultrafiltrate was directly injected after a simple one-step dilution procedure. The method was validated according to FDA standards (FDA, 2001) in terms of sample stability, specificity, accuracy and precision. Linearity was demonstrated in a concentration range of 0.2-20 µg/ml. In order to determine the unbound fraction of linezolid, ultrafiltrate samples (Centrifree®, Millipore, Eschborn, Germany, weight cut-off 30 kDa) were measured according to a previously established method (Buerger et al., 2006).

Pharmacokinetic data analysis: PK model building for describing the typical concentration-time profile and characterizing the variability in the population was performed using the nonlinear mixed-effects modeling approach implemented in NONMEM™ (Globomax, Version V, Level 1.1, 1998). First-order conditional estimation with interaction was used as estimation method. In a stepwise approach, first a structural model was developed. Afterwards, interindividual and residual variability were characterized. The model was parameterized in terms of clearances and distribution volumes using the PREDPP subroutine ADVAN 6 supplied in NONMEM™. Although the number of included individuals in the data analysis was not very large it was sufficient to estimate the included number of model parameters as the characteristic of combining data from different sources is one major advantage of the population analysis technique compared to individual PK parameter estimation. The reason for this is that the information of all subjects is interchangeably used to estimate population and also individual parameters.

Interindividual variability in model parameters was modeled using an exponential error term according to the equation

\[ P_{ki} = \theta_k \cdot e^{\eta_k} \]  

(1)
where \( P_{ki} \) denotes the value of the parameter \( k \) from the individual \( i \) (= individual parameter).

The model assumed that all \( P_{ki} \) were log-normally distributed. \( \theta_k \) is the typical value of the population parameter \( k \) and \( \eta_{ki} \) is the difference between the natural logarithm of \( P_{ki} \) and \( \theta_k \).

If individual parameters had to be confined to values between 0 and 1 this error model was modified according to the equation

\[
P_{ki} = \frac{e^{\ln(\theta_k/(1-\theta_k)) + \eta_{ki}}}{1 + e^{\ln(\theta_k/(1-\theta_k)) + \eta_{ki}}}
\]

(2)

where \( \theta_k \) is the typical value of the population parameter \( k \) and \( \eta_{ki} = \ln \frac{P_{ki}}{1-P_{ki}} - \ln \frac{\theta_k}{1-\theta_k} \).

Due to the exponential term, in this equation the numerator can only take values larger than zero. If it approximates values close to zero, \( P_{ki} \) will approach a value of 0. In contrast, if the numerator yields values much larger than zero, \( P_{ki} \) will approach a value of 1.

Residual variability, that is the discrepancy between the individual observed \( (C_{obs,ij}) \) and the individual model-predicted \( (C_{pred,ij}) \) plasma concentrations, was expressed as a combined error model with a proportional component, \( \varepsilon_1 \), and an additive one, \( \varepsilon_2 \), as follows:

\[
C_{obs,ij} = C_{pred,ij} \cdot (1 + \varepsilon_{1,ij}) + \varepsilon_{2,ij}.
\]

(3)

\( \varepsilon_{ij} \) denotes the random deviation between the individual prediction and the observed concentration for each individual \( i \) at a certain time point \( j \). The random-effects parameters \( \eta \) and \( \epsilon \) were assumed to be symmetrically distributed with zero means and variances of \( \omega^2 \) and \( \sigma^2 \), respectively.

The model-building process was guided by analyzing the goodness of fit plots created with Xpose, version 3.104 (Jonsson and Karlsson, 1998), precision of parameter estimates and the objective function value (OFV) provided by NONMEM™. The latter was used for discrimination between hierarchical models in the likelihood ratio test (Bonate, 2005). The addition of a parameter was considered significant if the decrease in objective function value
was > 3.84, corresponding to a p-value of 0.05 (df = 1). Only models that converged successfully were considered and are presented here.

**Model evaluation:** For evaluation purposes it was attempted to fit population models to the data which had been described for linezolid in previous reports, i.e. a linear two-compartment model (Whitehouse et al., 2005) and a two-compartment model with parallel linear and Michaelis Menten elimination (Meagher et al., 2003). The competing models were evaluated using goodness of fit plots. Moreover, the predictive performances of all models were compared by applying a predictive check. For this purpose, 1000 new individual concentration-time profiles were simulated in NONMEM™ based on the parameter estimates from the final models. The simulation was performed for intravenous dosing only. From all simulated concentrations the median and the 5th and 95th quantile were calculated for each time point using the software S-Plus (Insightful Corporation, Version 6.0 Professional Release 2, 2001). Based on these calculations, the 90% prediction interval and the median concentration-time course were presented graphically. All measured unbound linezolid concentrations were then compared to the corresponding 90% prediction intervals.

**Results**

**Available data:** From the clinical trial, 1176 observation records of unbound linezolid were available for model building purposes. All 34 individuals were studied at visit 1. Multiple dose data was available from 82.4% of the study population (90%, 88% and 75% for healthy volunteers, septic patients and patients with septic shock, respectively). The number of observations per subject covered a range of 16–20 and 25–40 for individuals studied only at visit 1 and individuals studied at visit 1 and 2, respectively.

**Concentration-time profiles:** The semi-logarithmic geometric mean concentration-time profiles of healthy volunteers and patients after single and multiple dosing are shown in figure 2. In all individual profiles depicting unbound plasma data after intravenous linezolid administration two disposition phases, i.e. two slopes could be observed that presumably
belonged to an early predominant distribution and a later predominant elimination phase. In contrast, after oral dosing (healthy volunteers) the early distribution phase did not become apparent. When comparing the plots obtained after single and multiple dosing a change in the disposition of linezolid was visible in both populations studied, i.e. the slope of the elimination phase became shallower over time, which indicated nonlinearity in the PK of linezolid.

**Population PK model:** In the population PK approach, all unbound plasma concentration data were fitted simultaneously. This approach was feasible because the main characteristics in the concentration-time profiles were similar in both population subgroups, indicating similar pharmacokinetics. Several structural PK models, comprising one-, two-, and three-compartmental structures, were investigated. Initial model building activities confirmed that a two-compartment model should provide the basis for all further model building activities (OFV=1476). The following fixed-effects parameters were included into the model: clearance [CL], volumes of distribution [V2, V3], intercompartmental clearance [Q] and absorption rate constant [KA]. A fixed absorption lag-time after oral dosing [ALAG1] was included for three selected individuals only after visual inspection of the concentration-time profiles as the available data was not sufficient to estimate ALAG1 for all individuals. Bioavailability was not included in the model as it was reported to be 100% and initial modeling activities confirmed this value. However, a simple 2-compartment model was not sufficient to account for the observed PK nonlinearity. Therefore, it was explored if PK nonlinearity could be explained by parallel linear and Michaelis Menten kinetics, an inhibition of clearance over time or a clearance dependent on the concentration in an inhibition compartment, similar to an effect compartmental approach. The model using parallel linear and Michaelis Menten kinetics led to a significant drop in OFV compared to the model using linear elimination only (OFV=1029; ∆OFV=447). As the two models were nested it was concluded that the model using parallel linear and Michaelis Menten kinetics was superior to the one using linear elimination only. However, goodness of fit plots revealed that the model
using parallel linear and Michaelis Menten elimination still contained some misspecifications. Especially small concentrations were overestimated. The misspecifications were reduced when incorporating a non-mechanistic inhibition of clearance over time, i.e. the decrease in clearance was a direct function of time. Finally, in a last step this inhibition over time was more mechanistically included in the model by introducing an additional inhibition compartment (OFV=750, ∆OFV=-726 compared to the model using linear elimination only; models were nested). Depending on the concentration in this empirical compartment CL from the central compartment was inhibited. In course of time, CL was allowed to take values between 0 and 100% of the original value estimated for the time of the first linezolid administration. The fraction of CL which could not be inhibited was represented by the parameter RCLF (remaining CL fraction). Thus, a RCLF estimate of 0 corresponded to a clearance inhibition of 100% whereas a RCLF estimate of 1 represented no clearance inhibition. In addition, to describe the time course of CL inhibition a rate constant into the inhibition compartment [KIC] and the concentration in the inhibition compartment yielding 50% of CL inhibition [IC50, fixed] were included as additional model parameters. A schematic illustration of the model is presented in figure 3. The following differential equations describing the change in amount per time for each compartment (1: absorption compartment, 2: central compartment, 3. peripheral compartment, 4: inhibition compartment) were applied:

\[
\begin{align*}
\frac{dA_1}{dt} &= -KA \cdot A_1 \\
\frac{dA_2}{dt} &= KA \cdot A_1 - \frac{Q}{V_2} \cdot A_2 + \frac{Q}{V_3} \cdot A_3 - \frac{CL}{V_2} \cdot INH \cdot A_2 \\
\frac{dA_3}{dt} &= \frac{Q}{V_2} \cdot A_2 - \frac{Q}{V_3} \cdot A_3 \\
\frac{dA_4}{dt} &= KIC \cdot \left( \frac{A_2}{V_2} - A_4 \right)
\end{align*}
\]

with \( INH = RCLF + (1 - RCLF) \cdot (1 - A_4 / (IC50 + A_4)) \). Interindividual variability (IIV) was incorporated for CL, V2, V3 and KA based on equation (1) and for RCLF according to equation (2). IIV was moderate to high with coefficients of
variation (CV) of 42% [ωCL], 40% [ωV2], 35% [ωV3] and 72% [ωKA]. A smaller number of individuals might generally lead to higher interindividual variability (upward bias) but our parameter estimates were not in an unexpected range. As a result of the different individual parameter distribution of RCLF compared to the other PK parameters (U-shaped, figure 4) it may not be appropriate to calculate a CV for RCLF. However, its IIV can be described by the 95% confidence interval which covered the range of 0.00374-0.99963. All parameters were estimated with acceptable precision as most relative standard errors were below 50%. Parameter estimates of the final model are presented in table 1.

**Goodness of fit:** The goodness of fit of the final model and the competing models is presented in figure 5. Inspection of the goodness of fit plots of the model with linear elimination (figure 5 A) showing population predictions against observed concentrations revealed some degree of model misspecification. Particularly, observed low values were overestimated by the model. This became even more apparent in a plot with log time vs. weighted residuals which showed an explicit trend in the early time phase. Values of the elimination phase after single dosing were overestimated, which further supported the assumption that nonlinearity in linezolid PK was involved, changing its disposition in the course of therapy. The second competing model included both a linear and a Michaelis Menten elimination pathway (figure 5 B). The trend that was visible in the model with linear elimination was less pronounced, however it was still distinguishable. Furthermore, low concentrations at the start of linezolid treatment were overestimated. On the contrary, the goodness of fit plot of the final model (figure 5 C) revealed that the trend of overestimation which could be observed in the model with linear and the model with parallel linear and Michaelis Menten elimination was eliminated. Furthermore, in contrast to figure 5 A and B in the plot showing population predictions against observed concentrations all concentrations were uniformly spread around the line of unity, indicating that the data was well described by the model.
In one of the patients extremely high concentration values were observed after multiple dosing (see figures 5 and 6). Therefore, it was investigated if this individual influenced parameter values by case deletion diagnostics. The exclusion of this subject led to acceptable maximum parameter changes of less than 7% compared to the reported final values in table 1 (maximum change for $\omega V3$: 6.96%).

**Predictive check:** The predictive check was performed to evaluate which of the investigated models adequately predicted the observed concentration-time profiles of unbound linezolid. After the first dose an inhibition of clearance developing over time would not be distinctly observable. Therefore, the early median concentration-time course was well captured by all investigated models. However, the best predictions of single dose data were obtained with the inhibition compartment model on the condition that $\omega RCLF$ was set to zero, i.e. under the assumption that clearance would be inhibited to the same extent in every individual, i.e. every individual RCLF value would be the same. This assumption had to be implemented in the predictive check due to the special coding for interindividual variability on the parameter RCLF as individual parameter distribution according to equation 2 resulted in a U-shape (figure 4).

The results of the predictive check after intravenous multiple dosing are presented in figure 6. Black lines represent the simulated median concentration-time profile whereas grey lines represent the 5th and 95th quantile and enclose the 90% prediction interval. The black symbols correspond to the measured concentrations after multiple dosing. Here, the differences in the predictive performance of the models were pronounced. In both the model with linear as well as in the model with parallel linear and Michaelis Menten elimination the 90% prediction interval was too wide and the predicted median concentration-time course was highly overpredicted. Again, the best predictions could be obtained by using the inhibition compartment model on the condition that $\omega RCLF$ was set to zero. For this model the simulated median concentration-time profile as well as the 90% prediction interval closely reflected the observed concentrations.
Discussion

In this analysis, the PK of linezolid was successfully described by a two-compartment model with linear elimination. However, this linear CL could be inhibited dependent on the concentration in an empirical inhibition compartment, resulting in an overall change in CL over time. This model structure was superior to all other investigated published and own alternatives with respect to goodness of fit and model stability. The population PK of linezolid had been described in the literature prior to this investigation. A linear one-compartment model underestimated all concentrations (Whitehouse et al., 2005), most pronounced for $C_{\text{max}}$ values. Using a linear two-compartment model, the obtained PK parameters were not in accordance with any other investigations, e.g. the distribution volume was estimated to be 284 l (Whitehouse et al., 2005) whereas others reported a volume of distribution of 40-50 l (Diekema and Jones, 2001). This led to the conclusion that both a classical linear one- and two-compartment model were not sufficient to describe linezolid PK, although stated otherwise by the authors (Whitehouse et al., 2005). This conclusion was confirmed by own investigations. Fitting a two-compartment model with linear elimination to the data resulted in model misspecifications which became apparent when plotting weighted residuals against the logarithm of time. As a result of the underlying assumption of a constant CL for single and multiple dose data concentrations were distinctly overestimated in the drug elimination phase of the first dose. Examination of the individual concentration-time profiles revealed a change in the disposition of linezolid when comparing concentration-time profiles obtained after single dosing to those obtained after multiple dosing, suggesting PK nonlinearity which might presumably be attributed to CL.

Antal et al. (Antal et al., 2000) reported a one-compartment model with parallel linear and Michaelis Menten elimination. Clearance estimation resulted in values of 10.99, 6.96, and 4.51 l/h for concentrations of 1.02, 5.86 and 13.94 µg/ml, respectively. Meagher et al. developed a two-compartment model with parallel linear and Michaelis Menten elimination
A combination of the linear and nonlinear elimination resulted in a mean average total clearance of 6.85 l/h/65 kg. A model using only a Michaelis Menten process for the description of linezolid clearance was presented by Beringer et al. (Beringer et al., 2005) for the description of single dose data. Its performance was compared to that of a linear model. The maximum metabolic rate $V_{\text{max}}$ and the Michaelis Menten constant $K_{\text{m}}$ were in the same range as those estimated before (Meagher et al., 2003), resulting in a clearance between 4.5-22.1 l/h for concentrations between 0-14 µg/ml. In the linear model clearance was estimated to be 11.2 l/h (Beringer et al., 2005).

Although the presented studies all reported to have successfully described the PK of linezolid the results indicated that the use of Michaelis Menten elimination might not be suitable. In such a case, one would expect a decrease in clearance with increasing doses. However, in the study performed by Antal et al. (Antal et al., 2000) a plot showing weight-normalized clearance against weight-normalized dose revealed that clearance varied randomly with differing doses. Meagher et al. (Meagher et al., 2003) reported that clearance seemed to change with an increasing number of doses. Unfortunately, they did not report if the change in clearance also became apparent in subjects who had similar concentrations after single dosing and at steady state. However, they described an apparent change in $V_{\text{max}}$ and $K_{\text{m}}$ with increasing doses when applying a model with sole Michaelis Menten elimination. This might also indicate that this type of model still contained some misspecifications. Finally, the model presented by Beringer et al. (Beringer et al., 2005) was based on single dose data and every subject was given the same dose. The authors concluded that the model with linear elimination and the one with Michaelis Menten elimination performed equally well. However, as the model did not include multiple dose data and different drug amounts the performance of the two competing models might not have been completely assessable. Finally, own investigations based on the total dataset described in this investigation and on reduced data sets containing only one population subgroup (healthy volunteers or patients) revealed that linezolid disposition changed over time even if $C_{\text{max}}$ values after single and multiple dosing...
were almost equal, as seen in 26.5% of the subjects (total dataset). These observations cannot be explained by saturable Michaelis Menten kinetics. When fitting a model with parallel linear and Michaelis Menten elimination to the available data the model misspecification became apparent when plotting weighted residuals against logarithm of time (figure 5). Although the trend was reduced in comparison to the model with linear elimination, concentrations were overestimated in the drug elimination phase of the first dose. Thus, a direct concentration dependence could be excluded from further considerations.

A mechanistic explanation for the observed nonlinearity may be given by an auto-inhibition of clearance over time although this had so far not been described for linezolid. The auto-inhibition was incorporated by using an empirical inhibition compartment. This approach resulted in an improved model. The trend previously observed when plotting weighted residuals against the logarithm of time was eliminated. Most of the estimated parameters corresponded to those presented in the literature before. However, the estimate for clearance exceeded that of other investigations. Stalker et al. observed a clearance of 7.38 l/h after multiple administration of 625 mg of linezolid (Stalker and Jungbluth, 2003). In order to correctly interpret the observed higher clearance value one has to consider that the clearance estimated by the model presented here was the maximum possible value. It would only apply to the first dose of linezolid when inhibition was negligible. Considering this, the estimated value of 11.1 l/h well corresponded to e.g. the value of 11.2 l/h estimated by a linear model after single dose administration (Beringer et al., 2005). The decrease in clearance was triggered by the parameter RCLF. The estimate of RCLF revealed that clearance could be inhibited to 76.4% of its original value. Consequently, population clearance changed to 8.48 l/h over time. This value was higher than some of the other values mentioned above. Nevertheless, this model also led to some individual clearance values in the range of 4 l/h in course of time. Moreover, the presented value was a result of clearance not being a direct function of concentration as in the Michaelis Menten model. The resulting values might thus not be directly comparable.
Finally, the best predictions were obtained by using the inhibition compartment model while the other models tended to overpredict concentrations after multiple dosing. Although the predictive check for the inhibition compartment model was performed using a model simplification by assuming the same extent of clearance inhibition in every individual, the results indicate that the presented model was superior to those that have been presented in the literature before.

From a mechanistic perspective, the inhibition of linezolid clearance might result from changes in linezolid metabolism. One of the major linezolid metabolites is the hydroxyethyl glycine metabolite (see figure 1). It is produced via formation of its precursor hydroxylinezolid (Feenstra et al., 1998). The production of hydroxylinezolid was demonstrated to be dependent on NADPH (Wynalda et al., 2000). Secondly, it was hypothesized that linezolid inhibited mitochondrial activity (Palenzuela et al., 2005), and only recently it was confirmed that linezolid induced a dose- and time-dependent decrease of mitochondrial respiratory chain enzyme activity at therapeutic concentrations (De Vriese et al., 2006). The formation of NADPH is connected to the respiratory chain enzyme activity by glycolysis and the pentose phosphate pathway. The ATP required for glycolysis is a product of the citric acid cycle and oxidative phosphorylation which account for 95% of all energy used by aerobic human cells (Berg et al., 2003). In consequence, the hypothesis can be generated that linezolid inhibits its own metabolism via inhibition of the mitochondrial respiratory chain enzyme activity (figure 7). It has been reported that 40% of the administered linezolid dose are eliminated as the hydroxyethyl glycine metabolite via the formation of hydroxylinezolid (Slatter et al., 2001). The final model estimated an inhibitable clearance fraction of 23.6%. The metabolic capacity of this pathway would thus be inhibited by slightly more than 50%. It is highly probable that the observed PK nonlinearity resulted from the clearance inhibition which was due to the inhibition of the formation of the major linezolid metabolite. This hypothesis offers a mechanistic explanation for the presented empirical PK model.
Further modeling activities might aim at using an indirect response model (Mager et al., 2003). After identifying covariate relations the developed model might be used for practical purposes in clinical treatment, i.e. for an individualized antimicrobial therapy which might also prevent further development of antimicrobial resistance.
Acknowledgements:
The authors acknowledge Karsten Roettger, Dieter Lang, Guido Finck, Dr. Anna Santarelli, Dr. Michael Toursarkissian, Dr. Pejman Dehghanyar, Dr. Markus Zeitlinger, Dr. Florian Islinger, Katharina Kuester, Christian Scheerans, Sabrina Albrecht, Stefanie Mendow and Sven Gruetzmann for contributing to the conduction of the clinical trial or technical assistance.
References


Pharmacia (2005) Zyvoxid® product information for healthcare professionals, Pharmacia GmbH Germany, Karlsruhe.


Footnotes:

a) Financial support: ‘Berliner Program zur Foerderung der Chancengleichheit fuer Frauen in Forschung und Lehre’, Berlin, Germany, and Dr. August and Dr. Anni Lesmueller foundation, Munich, Germany. Pharmacia is acknowledged for providing financial support for study materials.

b) Prof. Dr. Charlotte Kloft
Martin-Luther-Universitaet Halle-Wittenberg
Faculty of Pharmacy
Dept. Clinical Pharmacy
Wolfgang-Langenbeck-Str. 4
D-06120 Halle
Email: charlotte.kloft@pharmazie.uni-halle.de

c) Freie Universitaet Berlin, Institute of Pharmacy, Berlin, Germany (N.P., C.B., C.K.)
University School of Medicine, Vienna, Austria (C.J.)
DRK Kliniken Berlin Westend, Berlin, Germany (S.K.)
Martin-Luther-Universitaet Halle-Wittenberg, Faculty of Pharmacy, Halle, Germany (C.K.)
Legends for figures:

FIG. 1: Major metabolic pathways of linezolid

FIG. 2: Semi-logarithmic concentration-time profiles of healthy volunteers (left) and patients (right) after single (filled squares) and multiple dosing (open squares) of 600 mg linezolid bid. Volunteers received their first dose as an intravenous infusion and all subsequent doses orally while patients were only dosed intravenously. Data is presented as geometric mean + standard deviation. The x-axis presents the relative time elapsed after the last dose at study visit 1 and 2, respectively, i.e. drug administration is time = 0 (based on Buerger et al., 2006 and Thallinger C, Buerger C, Plock N, Klijucar S, Kloft C, Joukhadar C. Linezolid concentrations in soft tissues in healthy volunteers, patients presenting sepsis and septic shock, in preparation).

FIG. 3: Final structure of the population pharmacokinetic model for unbound linezolid plasma concentrations. Clearance (CL) is inhibited based on the concentration in an empirical inhibition compartment. A4 corresponds to the concentration in the inhibition compartment. INH corresponds to \([RCLF+(1-RCLF)\cdot((1-A4)/(IC50+A4))];\) KA: absorption rate constant; ALAG: absorption lag-time; V2: central volume of distribution; Q: intercompartmental clearance; V3: peripheral volume of distribution; KIC: rate constant into inhibition compartment; RCLF: remaining CL fraction, i.e. fraction of clearance which cannot be inhibited; IC50: concentration in the inhibition compartment yielding 50% of maximum clearance inhibition.

FIG. 4: Distribution of individual values of RCLF (simulation of 10000 values). In contrast to the normal distributions of the other PK parameters individual values of RCLF form a U-shape.

FIG. 5: Goodness of fit plots of the final model and previously published, competing models. Filled circles: healthy volunteers, empty circles: patients. A: model with linear elimination; B: model with both a linear and a Michaelis Menten elimination pathway; C: inhibition compartment model

FIG. 6: Predictive check for data obtained after intravenous multiple dosing. A: model with linear elimination; B: model with both a linear and a Michaelis Menten elimination pathway; C: inhibition compartment model, \(\omega RCLF\) was set to zero; Black lines represent simulated median concentration-time profile whereas grey lines represent 5th and 95th quantile and enclose the 90% prediction interval. Black symbols correspond to the measured concentrations.

FIG. 7: Proposed mechanism of linezolid clearance inhibition. Linezolid may inhibit its own metabolism via inhibition of the mitochondrial respiratory chain enzyme activity.
<table>
<thead>
<tr>
<th>Model parameter</th>
<th>Estimate</th>
<th>RSE (^a) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL [L/h]</td>
<td>11.1</td>
<td>7.84</td>
</tr>
<tr>
<td>V2 [L]</td>
<td>20.0</td>
<td>8.15</td>
</tr>
<tr>
<td>Q [L/h]</td>
<td>75.0</td>
<td>8.55</td>
</tr>
<tr>
<td>V3 [L]</td>
<td>28.9</td>
<td>7.99</td>
</tr>
<tr>
<td>KA [1/h]</td>
<td>1.81</td>
<td>25.9</td>
</tr>
<tr>
<td>ALAG1 [h]</td>
<td>1.27 FIX</td>
<td>-</td>
</tr>
<tr>
<td>RCLF [h]</td>
<td>0.764</td>
<td>14.3</td>
</tr>
<tr>
<td>KIC [1/h]</td>
<td>0.0019</td>
<td>5.19</td>
</tr>
<tr>
<td>IC50 [mg/L]</td>
<td>0.1 FIX</td>
<td>-</td>
</tr>
</tbody>
</table>

**Interindividual variability**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\omega_{CL})</td>
<td>41.7</td>
<td>22.2</td>
</tr>
<tr>
<td>(\omega_{V2})</td>
<td>40.1</td>
<td>22.3</td>
</tr>
<tr>
<td>(\omega_{V3})</td>
<td>34.8</td>
<td>31.0</td>
</tr>
<tr>
<td>(\omega_{KA})</td>
<td>72.4</td>
<td>57.1</td>
</tr>
<tr>
<td>(\omega^2_{RCLF})</td>
<td>11.8</td>
<td>52.5</td>
</tr>
<tr>
<td>Corr_CL/V3 (^d)</td>
<td>0.383</td>
<td>49.5</td>
</tr>
<tr>
<td>Corr_V2/RCLF (^e)</td>
<td>0.384</td>
<td>62.2</td>
</tr>
</tbody>
</table>

**Residual Variability**

<table>
<thead>
<tr>
<th>Variability</th>
<th>Estimate</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\sigma_{proportional})</td>
<td>8.96</td>
<td>9.50</td>
</tr>
<tr>
<td>(\sigma_{additive}) [mg/L]</td>
<td>0.292</td>
<td>31.8</td>
</tr>
</tbody>
</table>

\(^a\): relative standard error; \(^b\): coefficient of variation; \(^c\): Standard error given on the variance scale; \(^d\): correlation between CL and V3; \(^e\): correlation between V2 and RCLF; \(^f\): Standard error of the covariance estimate
Figure 1

linezolid → aminoethoxyacetic acid metabolite
(metabolite A)

\[
\text{hydroxylinezolid (unstable)} \quad \text{hydroxyethyl glycine metabolite (metabolite B)}
\]
Figure 2

![Graphs showing concentration over time for healthy volunteers and patients.](#)
Figure 3
Figure 5
Figure 6

A

B

C

Concentration [μg/mL]

Time after dose [h]

Concentration [μg/mL]

Time after dose [h]

Concentration [μg/mL]

Time after dose [h]
Figure 7

Respiratory chain
(citric acid cycle + oxidative phosphorylation)

Linezolid

ATP

ADP

Glucose-6-phosphate

NADP

NADPH

Hydroxylnezolid

Linezolid

Ribulose-5-phosphate