

Target Site Pharmacokinetics of Linezolid After Single and Multiple Doses in Diabetic Patients With Soft Tissue Infection

The Journal of Clinical Pharmacology
54(9) 1058–1062
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Clinical Pharmacology
DOI: 10.1002/jcph.296

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Abstract

The underlying pathology of diabetic wounds, i.e. impairment of macro- and microcirculation, might also impact target site penetration of antibacterial drugs. To compare tissue concentrations of linezolid in infected and not infected tissue 10 patients suffering from type 2 diabetes with foot infection were included in the study. Tissue penetration of linezolid was assessed using in vivo microdialysis at the site of infection as well as in non-inflamed subcutaneous adipose tissue. All patients were investigated after receiving a single dose of linezolid and five patients in addition at steady state. After a single dose of linezolid significantly higher area under the concentration vs. time curve over 8 hours (AUC_{0-8}) and maximum concentrations (C_{max})-values were observed in plasma (65.5 ± 21.2 mg^{*}h/L and 16.4 ± 4.6 mg/L) as compared to inflamed (36.3 ± 22.9 mg^{*}h/L and 6.6 ± 3.6 mg/L) and non-inflamed tissue (33.0 ± 17.7 mg^{*}h/L and 6.7 ± 3.6 mg/L). Multiple administrations of linezolid led to disappearance of significant differences in C_{max} and AUC_{0-8} between plasma, inflamed, and non-inflamed tissue. Approximately 2-fold increase of C_{max} and AUC_{0-8} -values in tissue was observed at steady state as compared to the first administration. Penetration of linezolid is not impaired in diabetic foot infection but equilibrium between plasma and tissue might be delayed.

Keywords

linezolid, tissue, pharmacokinetics, inflammation

The increasing prevalence of bacterial resistance necessitates appropriate use of antimicrobial agents, which can be only achieved by a better understanding of their pharmacokinetics (PK) and pharmacodynamics (PD). In particular, soft-tissue infections should be treated with antibiotics that penetrate well not only healthy, but also infected tissues. In clinical practice, the treatment of soft-tissue infections in patients with diabetes poses a real challenge. The reduced perfusion in the context of accompanying angiopathy impairs drug concentrations at the site of the ulcer and the risk for developing resistant strains increases with ongoing long-term antimicrobial therapy. Diabetic foot infections account among the most severe diabetic complications, as they delay healing, might cause osteomyelitis and septicemia, and are the main cause of non-traumatic foot amputations.^{1,2}

Linezolid is increasingly used in the antimicrobial therapy of infected wounds in patients with diabetes. Linezolid belongs to the oxazolidinone family, is highly active against antibiotic-resistant Gram-positive organisms including glycopeptide-resistant gram-positive bacteria and methicillin resistant *Staphylococcus aureus* (MRSA) and is approved for the treatment of complicated skin and soft-tissue infections.³ At steady state, its penetration into the

infected wound tissue was described to be good and comparable with its penetration in normal tissues.⁴ However, achieving of appropriate target site concentrations already after the first dose is considered beneficial for avoiding

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Submitted for publication 14 November 2013; accepted 23 March 2014.

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development of bacterial resistance, and hitting “hard and early” has become a paradigm in antimicrobial therapy.⁵

This study aimed to assess whether currently employed therapy regimens with linezolid provide effective antimicrobial concentrations at the site of diabetic soft tissue infection after the first dose and to compare them with concentrations at steady state. Target site PK of linezolid was assessed and compared to linezolid PK in plasma and non-inflamed subcutaneous adipose tissue.

Patients and Methods

Patients

The protocol was performed at the University Hospital of Vienna in agreement with the principles of Good Clinical Practice, the Declaration of Helsinki in its current version and the Austrian drug law. The study was approved by the institutional review board of the Medical University of Vienna and written informed consent was obtained from all study participants prior to inclusion in the study. Ten patients with diabetes mellitus type 2 and skin or soft tissue infections of the foot were enrolled into the study and received linezolid for therapeutic reasons. For all 10 patients, PK of linezolid (Zyvoxid[®] 600 mg in ready-to-use infusion bags (300 mL), Pfizer, New York, USA) was investigated after a single dose (SD) of linezolid. The linezolid dosage for the 5 patients receiving multiple doses was 600 mg every 12 hours for at least 3 days before blood and tissue sampling ensued. The doses of linezolid administered in the present study correspond to the current standard dosages, as recommended by the Summary of Product Characteristics; all doses were administered as short time infusion over 30 min.

Microdialysis Procedure

Microdialysis is based on sampling of analytes from the extracellular space by means of a semi-permeable membrane at the tip of a microdialysis probe. Once the probe is implanted into the tissue, substances present in the extracellular fluid are filtered by diffusion out of the extracellular fluid into the probe, and can be later detected in the collected perfusion medium.⁶

In the present study, 2 microdialysis-probes (CMA 63[®] microdialysis probe, cut-off 20,000 Da, CMA, Solna, Sweden) were inserted at each study day, 1 probe at the border of the soft tissue infection (caution was taken not to place it directly into an ulcer or purulent site), the other probe subcutaneously into non-inflamed subcutaneous adipose tissue of healthy thigh tissue. The probes were then constantly perfused with a physiological solution at 1.5 $\mu\text{L}/\text{min}$.

In order to limit discomfort to the subject due to immobility during microdialysis, the duration of the whole procedure was restricted to 12 hours (including placing of probes and the 1 hour equilibration, 8 hours sampling and 2 hours calibration periods). Sampling of

dialysates and venous blood were performed at baseline (30 minutes microdialysis baseline), at the end of the infusion, in 30 minutes intervals from 1 to 3 hours and in 60 minutes intervals up to 8 hours for PK analysis. Probes were calibrated according to retrodialysis (RD) methods as previously described.^{7,8} Approximately 4.5 mL venous blood for the measurement of linezolid plasma levels was collected into lithium–heparin tubes at each time point from the arm that was opposite to that was used for linezolid infusion. Blood samples were kept on ice for a maximum of 60 minutes and were centrifuged at +4°C and 3500 rpm for 10 minutes, cells were discharged and plasma was obtained. Microdialysates and plasma samples were stored at approximately –80°C until analysis.

Linezolid concentrations in microdialysates and plasma samples were analyzed using an established high performance liquid chromatography (HPLC) procedure with lower limits of quantification of 0.8 and 0.2 $\mu\text{g}/\text{mL}$ for microdialysate and plasma, respectively.⁹ Assay performance across the entire linezolid concentration range with respect to accuracy expressed as relative error was $\leq \pm 5.4\%$ and $\leq \pm 9.4\%$ and with respect to precision expressed as coefficient of variation was $\leq 6.1\%$ and $\leq 7.5\%$ for microdialysate and plasma, respectively.

PK and Statistical Analysis

Individual recovery values were assessed for each microdialysis probe and for each period and were used for calculating tissue concentration. The PK parameters maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), and terminal elimination half-life ($t_{1/2}$) were calculated using a commercially available computer program (Kinetic[®] 3.0, Innaphase, Philadelphia, PA, USA). Area under the concentration–time curve (AUC) from 0 to 8 hours (AUC_{0-8}) and AUC from 0 to 12 (AUC_{0-12}) were calculated from non-fitted data by employing the trapezoidal rule. In case of plasma for $\text{AUC}_{0-\text{inf}}$ individual extrapolation based on the last observed concentration and the elimination constant k_{el} was performed to allow for calculation of apparent total body clearance (CL) and apparent volume of distribution (V). To determine AUC_{0-12} , that is, the AUC for an standard dosing interval of 12 hours the concentration after 12 hours (C_{12}) was calculated by use of the formula ($C = C_{\text{last}} \cdot e^{-k_{\text{el}} \cdot t}$), where C represents the concentration at a defined time point, C_{last} is the last concentration measured, k_{el} is the elimination rate constant, and t is the time between the measurement of C_0 and the defined time point.

Statistical analysis was performed using the statistical software package SPSS release 15.0.1 (SPSS). Data are presented as mean + SD. The differences between the groups were tested using one-way paired ANOVA, followed by post hoc *t* tests when applicable. *P* values <0.05 were considered statistically significant.

Linezolid is administered twice daily; thus, to obtain a conservative estimation of AUC_{0-24} the respective AUC_{0-12} values for plasma, skeletal muscle tissue, adipose tissue were doubled. The estimated values were used for PK/PD calculations by dividing AUC_{0-24} over MIC_{90} values of relevant Gram positives pathogens obtained in the U.S.^{10,11}

Results and Discussion

Study participants were aged 59–81 years; their weight was 95 ± 8.9 kg, their BMI 32.7 ± 3.1 kg/m.² Mean HbA1c was $7.7 \pm 0.3\%$ and mean CRP 5 ± 2.2 mg/dL. All patients received a SD of linezolid (7 male and 3 female subjects), of whom 5 patients (3 male and 2 female subjects) also received multiple doses of linezolid. Both single- and multiple-dose administrations of linezolid were well tolerated and no drug or microdialysis related adverse events were observed. Mean linezolid recovery of microdialysis probes was $37 \pm 13\%$ for non-inflamed and 36 ± 14 for inflamed tissue. There was also no significant difference between recovery values obtained after the first dose and at steady state.

One single intravenous infusion of linezolid led to significantly higher AUC_{0-8} and C_{max} values in plasma (65.5 ± 21.2 mg h/L and 16.4 ± 4.6 mg/L) as compared to inflamed (36.3 ± 22.9 mg h/L and 6.6 ± 3.6 mg/L) and non-inflamed tissue (33.0 ± 17.7 mg h/L and 6.7 ± 3.6 mg/L) tissue (both $P < 0.001$, Table 1). There were no significant differences in linezolid concentrations and PK parameters between healthy and inflamed tissues after a single intravenous infusion of linezolid.

Multiple administrations of linezolid led to a disappearance of significant differences between plasma and healthy or inflamed tissue concentrations (Table 2). Compared to the first dose at steady state significantly increased linezolid concentrations (AUC_{0-8}) were observed in plasma ($P = 0.040$), but also in both healthy ($P = 0.005$) and inflamed tissues ($P = 0.043$), reaching approximately twofold increase of C_{max} and AUC_{0-8}

values in healthy and inflamed tissue at steady state as compared to SD administration.

The tissue to plasma ratio calculated by using the measured free AUC (AUC_{free}) in tissue and the estimated AUC_{free} in plasma is commonly considered as measure of tissue penetration of an antibiotic. When correcting for an average protein binding in plasma of 15%,⁴ after an SD mean tissue to plasma AUC_{free} ratios of 0.59 ± 0.25 and 0.63 ± 0.24 for healthy and inflamed tissue, respectively, were observed while the ratios increased to 0.98 ± 0.41 and 0.78 ± 0.23 at steady state. Therefore, the present study demonstrates that despite good plasma to tissue equilibration of linezolid was observed at steady state, after the initial dose significantly lower C_{max} and AUC values were observed in soft tissues when compared to plasma (Figure 1a and Table 1).

In good agreement with previous data from healthy subjects and diabetic patients, the PK parameters observed in the present study showed equilibration of linezolid between the blood compartment and interstitial space fluid of soft tissue at steady state (Figure 1b and Table 2).^{4,8} However, due to the fact that both SD and multiple dose data were generated within the same group of subjects the present study for the first time allows direct observation of the delay in equilibration between the 2 compartments in diabetic patients. As the discrepancy between tissue penetration after the first dose and at steady state was not observed in healthy volunteers, this finding can be deemed as indication than in case of diabetic patients pathologies like micro- and macrovascular disease might delay plasma to tissue equilibrium,⁸ while local inflammation seems to have no significant impact on PK of linezolid. Since previous studies performed infusion of linezolid over 1 hour compared to the 30 minutes infusions in the present study the observed higher C_{max} values in plasma were expected in the present study (at steady state 27.4 ± 21.2 as compared to 12.0 ± 3.7 for,⁴ however, the duration of infusion had no obvious impact on the C_{max} in tissues.

In animal models with linezolid the mean free plasma AUC_{0-24}/MIC ratio necessary for bacteriostasis was 19

Table 1. Pharmacokinetic Parameters of Linezolid in Plasma, in the Interstitium of Inflamed Soft Tissue of the Leg and of Healthy Soft Tissue of the Ipsilateral Thigh in 10 Patients with Diabetes Mellitus (Means \pm SD) After a Single Dose of 600 mg Linezolid

Parameter	Plasma SD (n = 10)	Healthy Tissue SD (n = 10)	Inflamed Tissue SD (n = 10)
C_{max} (mg/L)	16.4 ± 4.6	$6.7 \pm 3.6^*$	$6.6 \pm 3.6^*$
T_{max} (h)	0.5 ± 0.0	$2.3 \pm 1.6^*$	$3.0 \pm 1.9^*$
AUC_{0-8} (mg h/L)	65.5 ± 21.2	$33.0 \pm 17.7^*$	$36.3 \pm 22.9^*$
AUC_{0-12} (mg h/L)	82.3 ± 31.1	44.0 ± 23.7	48.1 ± 28.7
AUC_{0-24} (mg h/L)	164.5 ± 62.1	88.0 ± 47.3	96.2 ± 57.4
AUC_{0-inf} (mg h/L)	114.7 ± 62.4	ND	ND
$T_{1/2}$ (h)	6.0 ± 3.4	6.9 ± 6.3	5.6 ± 4.8
Clearance (L/h)	6.8 ± 3.7	ND	ND
V_z (L)	45.9 ± 10.8	ND	ND

Table 2. Pharmacokinetic Parameters of Linezolid in Plasma, in the Interstitium of Inflamed Soft Tissue of the Leg and of Healthy Soft Tissue of the Ipsilateral Thigh in 5 Patients With Diabetes Mellitus (Means \pm SD) After Multiple Doses of Linezolid 600 mg Twice Daily for at Least 3 Days

Parameter	Plasma MD (n = 5)	Healthy Tissue MD (n = 5)	Inflamed Tissue MD (n = 5)
C_{max} (mg/L)	27.4 \pm 21.2	14.9 \pm 4.8	12.7 \pm 5.3
T_{max} (h)	0.5 \pm 0.0	2.3 \pm 1.3*	2.5 \pm 1.1*
AUC ₀₋₈ (mg h/L)	100.8 \pm 37.9	77.3 \pm 23.7	66.1 \pm 26.5
AUC ₀₋₁₂ (mg h/L)	132.2 \pm 54.9	107.2 \pm 47.5	87.4 \pm 31.0
AUC ₀₋₂₄ (mg h/L)	264.3 \pm 109.8	214.4 \pm 94.9	174.8 \pm 62.0
AUC _{0-inf} (mg h/L)	189.8 \pm 99.7	ND	ND
$T_{1/2}$ (h)	6.6 \pm 3.5	10.4 \pm 8.3	4.1 \pm 0.8
Clearance (L/h)	4.1 \pm 2.4	ND	ND
V_z (L)	32.2 \pm 8.3	ND	ND

C_{max} , maximum concentration; T_{max} , time to maximum concentration; AUC, area under the concentration vs. time-curve for the indicated time interval; $T_{1/2}$, terminal half-time; V_z , volume of distribution.

For plasma total concentrations, for tissue free concentrations are presented.

* $P < 0.05$ when comparing parameters for plasma vs. tissue.

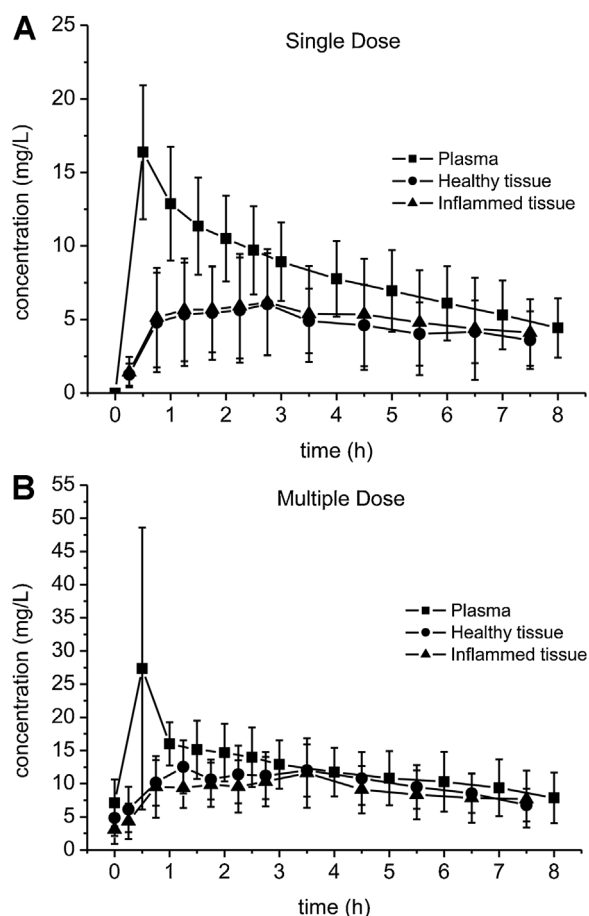


Figure 1. (A) Time versus concentration profile after a single dose of 600 mg linezolid in plasma (total concentration), healthy and inflamed tissue (free concentrations). Data are presented as mean \pm SD (n = 10). (B) Time versus concentration profile after multiple administrations of linezolid (600 mg twice daily for at least 3 days) in plasma (total concentration), healthy and inflamed tissue (free concentrations). Data are presented as mean \pm SD (n = 5).

while for achieving at least 1-log-unit kill reduction values of about 46.1 were needed.¹² Using AUC₀₋₂₄ values obtained in the present study, this threshold could be achieved or exceeded for MIC₉₀ values of up to 2 μ g/mL in all compartments already after SD (Table 1). Thereby MIC₉₀ values of oxacilin susceptible and resistant *S. aureus*, *Enterococcus faecalis* and *faecium* as well as different *Streptococci* spp. obtained from 2 U.S. susceptibility surveillance studies can be sufficiently covered.^{10,11} However, considering susceptibility breakpoints of linezolid for staphylococci and enterococci is ≤ 4 mg/L, this breakpoint would not be optimally covered for inflamed and healthy soft tissues after SD although it would be achieved at steady-state conditions. In addition, the considerably inter-individual variability of AUC₀₋₂₄ values observed would suggest that while tissue levels are sufficient in some patients more resistant strain would not be optimally targeted in others.

In order to avoid complications due to probe insertion, the microdialysis catheters were placed in peripheral zones around the inflamed area rather than in the infection or wound itself where pathophysiological deviations like decreased pH value and hypoxia might be present. According to the Biopharmaceutical Classification System (BCS) linezolid should have low permeability.¹³ However, both the excellent bioavailability of the drug as well as our findings contradict this theoretical classification system. In addition, linezolid was shown to maintain its full antimicrobial activity as well as bacterial penetration independent of pH, indicating our data might be used to predict activity directly in the focus of the infection.¹⁴

A prerequisite for extrapolating data between the last measured time point at 8 hours to the end of the dosing interval after 12 hours is linear PK during this time period. Indeed linezolid undergoes linear elimination at therapeutic dose ranges and in plasma the terminal phase of linezolid elimination is reached quickly after end of the infusion period.¹⁵ Appropriateness of the calculation and

linearity of linezolid PK is further highlighted by the good agreement of AUC_{0-inf} after SD (114.7 ± 62.4 mg h/L) and the AUC in the dosing interval at steady state (AUC_{0-12} of 132.2 ± 54.9 mg h/L). Together with the good temporal resolution of microdialysis, that is, 11 measured data points during the 8 hours observation period this suggests that extrapolation of data from 8 to 12 hours is reliable for all described compartments.

Doubling the resulting calculated AUC_{0-12} values in order to receive exposure over 24 hours for PK/PD calculation may be extremely conservative for the first dose of treatment, however, it does not compensate for potential circadian differences in PKs.¹⁶ As all patients started their investigations with the morning dose of linezolid interpretation of data has to be done with utmost caution. A further limitation of the present study is the low sample size; in particular only 5 patients were investigated under steady-state conditions. However, data at steady-state conditions were already previously described and showed good agreement with the present study.¹⁷

Soft-tissue infections in diabetic patients, such as foot ulcers, are extremely difficult to treat and often result in chronic infections.¹⁸ Despite the good antimicrobial activity of linezolid against all major Gram-positive underlying pathogens, linezolid should be only used if suspicion for MRSA is high, in which case it may be considered as first line therapy.¹⁹ Therefore, one might consider it even more important from a PK/PD perspective to use this antibiotic correctly starting with the first administration, since sub-inhibitory concentrations might accelerate development of resistance.²⁰ The concept of a loading dose, as previously established for other antibiotics like levofloxacin, should be considered in this case. However, also due to the limited sample size our mainly descriptive data can only be considered hypothesis generating and should be combined with other data on linezolid tissue PK at a broader base for population PK and Monte-Carlo simulations. Ultimately such a concept will have to be evaluated clinically under consideration of safety and tolerability aspects.

Declaration of Conflicting Interests

The authors declare that they have no financial or ethical conflict of interest in connection with this paper.

Funding

This study was financially supported by Pfizer. Pfizer had no influence of concept of the study or interpretation of data.

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