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Short communication

Linezolid concentrations in infected soft tissue and bone following repetitive doses in diabetic patients with bacterial foot infections☆

Friederike Traunmüller[a,b,1], Michael V. Schintler[b,1], Stephan Spendel[b], Martin Popovic[a,c], Oliver Mauric[a], Erwin Scharnagl[b], Christian Joukhadar[a,b,d,e,*]

[a] J&P Medical Research Ltd., Vienna, Austria
[b] Department of Surgery, Division of Plastic Surgery, Medical University of Graz, Austria
[c] Department of Radiology, Division of Cardiovascular and Interventional Radiology, Medical University of Vienna, Austria
[d] Beth Israel Deaconess Medical Center, Boston, MA, USA
[e] Harvard Medical School, Boston, MA, USA

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ABSTRACT

The present study aimed at assessing unbound extracellular concentrations of linezolid in inflamed soft tissue and bone of diabetic patients suffering from severe bacterial foot infections. Linezolid was administered intravenously twice daily at a dosage of 600 mg. At steady-state conditions, the microdialysis technique was utilised to sample serially interstitial space fluid from inflamed subcutaneous adipose tissue and metatarsal bone from 0–8 h post dose in three representative patients. Mean peak concentrations of free linezolid in plasma, healthy subcutis, inflamed subcutis and cancellous bone were 16.6 ± 3.0, 15.5 ± 2.5, 15.8 ± 2.8 and 15.1 ± 4.1 mg/L, respectively. The degree of tissue penetration as expressed by the ratio of the area under the concentration–time curve of free linezolid from 0–12 h ($fAUC_{0-12}$) in tissue to the $fAUC_{0-12}$ in plasma was 1.32 ± 0.09, 1.12 ± 0.22 and 1.09 ± 0.11 for healthy subcutis, inflamed subcutis and bone, respectively. Based on currently available pharmacokinetic/pharmacodynamic targets, we conclude that linezolid administered at 600 mg twice daily may be considered an effective treatment in diabetic patients suffering from bacterial foot infection complicated by osteomyelitis.

1. Introduction

In patients with diabetes, serious complications such as peripheral neuropathy combined with bacterial foot infections account for a large number of hospital stays and are a major cause of non-traumatic amputations of the lower limb. Appropriate management of these complications often requires intravenous (i.v.) administration of potent antimicrobial agents supported by surgical intervention. However, measures like these require coordinated interdisciplinary work between surgeons, clinicians, clinical pharmacologists and microbiologists because of uncertainty regarding resistance rates, rapidly evolving outbreaks of multiresistant bacterial strains following long-term therapy with broad-spectrum antibiotics, and incomplete knowledge of drug tissue penetration. Thus, choosing the right antimicrobial agent is essential in these situations.

Linezolid has demonstrated potent in vitro activity against problematic bacteria such as meticillin-resistant *Staphylococcus aureus* (MRSA) and glycopeptide-resistant Gram-positive bacteria [1]. The ability of linezolid to penetrate into the extracellular space fluid of soft tissues is well documented in healthy volunteers and critically ill patients [2,3]. However, the rate and extent of penetration of linezolid into bone tissue is controversial in the medical literature [4–7].

Thus, in the present study we aimed to determine the ability of linezolid to penetrate into bone and the interstitium of inflamed subcutaneous adipose tissue in a small representative cohort of diabetic patients suffering from severe diabetic foot infection (DFI). Recently, the microdialysis technique, which was used in this study, was also employed to assess unbound concentrations of linezolid in cancellous bone tissue of healthy pigs as well as fosfomycin concentrations in healthy and inflamed tissue of diabetic patients presenting with bacterial foot infections complicated by osteomyelitis [7,8].

2. Subjects and methods

This study was performed at the Division of Plastic Surgery, Department of Surgery, Landeskrankenhaus Universitätsklinikum

☆ Data from this study will be presented as an abstract and poster at the 4th Austrian Conference on Infectious Diseases, 5–8 May 2010, Saalfelden, Austria.

* Corresponding author. Present address: J&P Medical Research Ltd., Auhofstrasse 15/8-9, 1130 Vienna, Austria. Tel.: +43 1 8760432 10; fax: +43 1 8760432 33.
E-mail address: christian.joukhadar@jp-medical-research.com (C. Joukhadar).

[1] These authors contributed equally to this work.

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Table 1
Main pharmacokinetic indices of linezolid in plasma and target tissues of diabetic foot infections following repetitive intravenous doses of 600 mg [median (range); $n = 3$].

Tissue	C_{max} (mg/L)	T_{max} (h)	$T_{1/2\beta}$ (h)	V_{ss} (L)	AUC_{0-24} (mg h/L)[a]	$fAUC_{0-24}$/MIC[b]
Plasma (total)	22.4 (15.2–26.6)	0.5	9.3 (7.2–11.1)	36.9 (30.6–47.5)	229.4 (198.6–331.7)	–
Plasma (free)	17.8 (12.5–19.6)	0.5	9.3 (7.2–11.1)	–	169.1 (162.7–263.2)	84.6 (81.4–131.6)
Subcutis (healthy)	13.9 (13.6–19.0)	2.5 (2.0–3.0)	8.8 (7.8–10.9)	–	245.3 (202.3–349.8)	122.6 (101.1–174.9)
Subcutis (inflamed)	17.4 (11.9–18.3)	2.0 (1.5–3.0)	9.3 (7.9–9.5)	–	210.9 (210.7–212.9)	105.4 (105.4–106.5)
Metatarsal bone	17.0 (9.3–18.9)	2.5 (1.5–2.5)	9.2 (8.1–12.2)	–	210.4 (165.6–266.0)	105.2 (82.8–133.0)

C_{max}, peak concentration; T_{max}, time to C_{max}; $T_{1/2\beta}$, half-life at β-phase; V_{ss}, volume of distribution at steady-state; AUC_{0-x}, area under the concentration–time curve from 0–x h; f, free; MIC, minimum inhibitory concentration.
[a] Calculated for twice daily administration ($AUC_{0-12} \times 2$).
[b] Example for meticillin-resistant *Staphylococcus aureus* (MRSA) (MIC = 2 mg/L).

Graz (State Hospital University Clinic of Graz, Graz, Austria). Analytical work was performed at the laboratory of J&P Medical Research Ltd. (Vienna, Austria).

2.1. Study subjects

Three male patients with type II diabetes [Caucasians aged 60–67 years, body mass index (BMI) 26.3–37.2 kg/m^2] presenting with deep-seated bacterial foot infections were included in the study after oral and written informed consent was obtained. The patients required surgical debridement with partial metatarsal bone resection and adjuvant systemic antimicrobial therapy. Exclusion criteria were known allergy to linezolid, renal dysfunction indicated by a creatinine clearance of <40 mL/min as estimated by the Cockcroft–Gault formula, and a history of neutropenia or thrombocytopenia. All patients had received non-invasive conservative treatment for DFI prior to enrolment into the study. During the conduct of the study, co-administration of antimicrobial agents or medications other than the study drug was permitted if medically indicated. All patients received at least six standard i.v. doses of linezolid (Zyvoxid™, 600 mg/300 mL solution for infusion; Pfizer Corp., Vienna, Austria) over ca. 30 min twice daily prior to microdialysis.

2.2. Microdialysis and sampling procedures

Microdialysis was performed as described in detail elsewhere [8]. Venous blood was collected at predefined time points from an indwelling i.v. catheter.

2.3. Chemical analysis and determination of plasma protein binding

Linezolid concentrations in plasma and microdialysates were measured by high-performance liquid chromatography (HPLC) with ultraviolet detection as described previously [9]. The lower limit of quantification was 0.05 mg/L both for plasma and microdialysate.

Individual values of plasma protein binding were determined in duplicate by the ultrafiltration method. In brief, aliquots of plasma samples were ultrafiltered using disposable centrifugal filter devices (Ultrafree-MC, molecular cut-off 5000 Da; Millipore, Bedford, MA) at 7500 × g for 30 min. Filtrates were analysed for free linezolid. The bound fraction was calculated by subtracting the free fraction from unity.

2.4. Pharmacokinetic analysis

Pharmacokinetic calculations were carried out using the commercially available computer software Kinetica version 3.0 (InnaPhase, Philadelphia, PA). For the concentrations at 12 h, the baseline steady-state concentrations were utilised. Areas under the concentration–time curve (AUC) were calculated by use of the linear trapezoidal rule.

3. Results

No adverse events related to the study drug or to the microdialysis procedure were observed. Key pharmacokinetic parameters of linezolid in healthy and inflamed subcutaneous adipose tissue and in metatarsal bone are summarised in Table 1. Pharmacokinetic profiles are depicted in Fig. 1.

The degree of tissue penetration as expressed by the free (f) tissue to plasma ratios of the AUC from 0–12 h ($fAUC_{0-12\ tissue}/fAUC_{0-12\ plasma}$) was 1.32 ± 0.09, 1.12 ± 0.22 and 1.09 ± 0.11 for healthy subcutis, inflamed subcutis and bone, respectively. Plasma protein binding of linezolid in the study subjects ranged from 18% to 26%.

4. Discussion

MRSA infections associated with unfavourable outcomes are becoming increasingly problematic in diabetic foot clinics [10]. As moderate-to-severe DFI is frequently linked to osteomyelitis of adjacent bones, the ability of certain antibiotic agents to penetrate bone tissue needs to be taken into consideration when aiming for successful treatment of DFI. However, the vast majority of presently available pharmacokinetic data regarding bone tissues focuses on tissue homogenates, which provide no information on the concentration–time course of the drug at the relevant site of most infections, i.e. the extracellular space fluid [7].

Against this background, we utilised the microdialysis technique to determine the pharmacokinetic profiles of unbound linezolid in inflamed subcutaneous adipose tissue and metatarsal

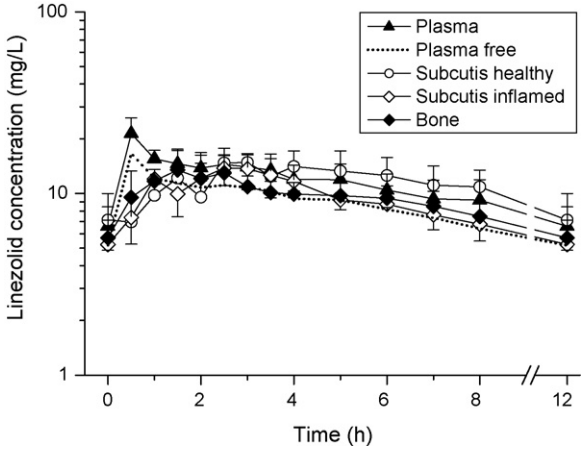

Fig. 1. Pharmacokinetic profiles of linezolid in plasma, soft tissues and metatarsal bone in diabetic foot infection following repetitive intravenous doses of 600 mg (mean ± standard deviation; $n = 3$).

bone. The key finding was that free linezolid in plasma equilibrates completely with soft tissues and bone within ca. 1 h after the start of infusion (Fig. 1). Tissue:plasma ratios of the $fAUC_{0-12}$ were ca. 1, indicating rapid and complete penetration of linezolid into the interstitial space fluid of investigated tissues. It is noteworthy to mention that for measurements in bone, the microdialysis probe was implanted into vital, macroscopically unaffected bone tissue located in close proximity to surgically resected sequestrating bone structures.

In addition, it was shown that inflammation did not affect interstitial concentrations of linezolid in soft tissues (Table 1; Fig. 1). This is in line with another study investigating concentrations of linezolid in perinecrotic wound tissue obtained by biopsy from patients with DFIs. In this study, mean tissue penetration of linezolid was 102% as determined 3 h after an oral dose of 600 mg [11]. On the other hand, in a study by Stein et al. [12], penetration of linezolid into soft tissues was only 18–78% (mean 51%) in diabetic patients. It may be speculated that a reduction in blood flow owing to peripheral vascular disease exerted a major impact on the plasma-to-tissue drug equilibration process [13,14]. Thus, in the present investigation, healthy subcutaneous adipose tissue of a more proximal region of the ipsilateral lower limb was used as reference tissue in order to eliminate potential bias from altered blood flow. We did not find any clinically relevant difference between concentrations of linezolid in healthy reference tissue and inflamed subcutaneous adipose tissue or bone (Table 1; Fig. 1).

The ratio of the $fAUC_{0-24}$ in plasma to the minimum inhibitory concentration (MIC) of the infecting pathogen ($fAUC_{0-24\ plasma}$/MIC) was shown to be highly predictive of antimicrobial killing and clinical success. In seriously ill patients, the target value of $fAUC_{0-24\ plasma}$/MIC to achieve clinical cure was determined to be 80–120 [15]. However, predictive pharmacokinetic/pharmacodynamic (PK/PD) targets for tissues are not yet established. In this study, the $fAUC_{0-24}$/MIC target of ca. 100 was attained for relevant pathogens with MICs < 4 mg/L in plasma and all tissues investigated [1].

In summary, based on PK/PD considerations, we conclude that free concentrations of linezolid are sufficient to cover soft tissue and bone infections with MRSA or other Gram-positive bacteria commonly isolated in diabetic patients with foot infections complicated by osteomyelitis.

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Competing interests: J&P Medical Research Ltd. is an independent international research institute basically operating according to the Public–Private Partnership concept. CJ is managing director of J&P Medical Research Ltd. and owns 100% options. CJ is also a consultant for pharmaceutical companies. All other authors declare no competing interests.

Ethical approval: The present study protocol was approved by the Ethics Committee of the Medical University of Graz (Austria).

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