Effect of severity of sepsis on tissue concentrations of linezolid

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Objectives: In the present study, we examined whether differences in the severity of sepsis translate to differences in the pharmacokinetic profile of linezolid in plasma and the interstitium of target tissues after a single intravenous dose of 600 mg by means of the microdialysis technique.

Patients and methods: A total of 24 patients were included in the trial. Sixteen patients suffered from septic shock and eight patients presented with severe sepsis. Sepsis was diagnosed and verified according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee. Historic data derived from a previous study determining the pharmacokinetic profiles of linezolid in tissues and plasma in young healthy volunteers served as controls.

Results: In the present study, the AUC for free linezolid from 0 to 24 h (fAUC0–24) ranged from 50 to 71 mg.h/L after single-dose administration in patients presenting with severe sepsis or septic shock. The mathematically extrapolated fAUC0–24 ranged from 100 to 146 mg.h/L for twice-daily administration and a dosing interval of 12 h. No statistically significant difference in key pharmacokinetic parameters was detected between patients suffering from severe sepsis and septic shock (P > 0.05).

Conclusions: These data indicated that the severity of sepsis has no substantial effect on the pharmacokinetic profile of linezolid in plasma and in the interstitium of soft tissues.

Keywords: pharmacokinetics, inflammation, single-dose

Introduction

The development of antimicrobial agents to treat infections caused by resistant Gram-positive bacteria, including penicillin-resistant Streptococcus pneumoniae, methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus faecium, is an area of intensive research. Linezolid belongs to a class of synthetic antibacterial agents called oxazolidinones and is approved for reserve treatment of serious infections caused by resistant Gram-positive aerobic and anaerobic pathogens. It is frequently administered to patients presenting with sepsis and septic shock.1,2

As most relevant infections develop in the extracellular space fluid of the tissue rather than in the blood, we used the microdialysis technique which is capable of assessing the interstitial concentrations of antibiotics in tissues.

In the present study, we investigated the pharmacokinetic profile of linezolid in the interstitium of subcutaneous adipose tissue and skeletal muscle after administration of a single intravenous dose of 600 mg to patients suffering from sepsis or septic shock. The rationale of this study is based on previous data, clearly demonstrating that the pharmacokinetics of distinct classes of antibiotics may be substantially affected in the interstitium of tissues and plasma in patients suffering from severe sepsis or septic shock.3,4

Materials and methods

The present multicentre study took place in selected intensive care units in university hospitals in Austria and Germany. One pivotal

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previous study performed by our study group was completed. This historic reference study published by Buerger et al. was initially performed in 11 patients with septic shock and in 1 patient presenting with severe sepsis. In order to be able to compare statistically the pharmacokinetic profiles in patients with severe sepsis with patients presenting with septic shock, we substantially increased the number of patients in both groups. Additionally, another study performed previously by our group using identical methods looked at the pharmacokinetic profile of linezolid in young healthy volunteers and served exclusively as controls. The study protocol was approved by the local Ethics Committees and was performed in accordance with the Declaration of Helsinki (1964) in the revised version of 2000 (Edinburgh), the Guidelines of the International Conference of Harmonization, the Good Clinical Practice Guidelines and the Austrian drug law. All healthy volunteers were given a detailed description of the study, and their written consent was obtained prior to their enrolment in the study. For comatose or sedated septic patients, written consent was sought as soon as it was medically possible. None of the patients received renal replacement therapy before and at least 48 h after performing the study.

Concentrations in interstitial space fluid of skeletal muscle and subcutaneous adipose tissue were determined by microdialysis. The principle of microdialysis has been described previously in detail. In brief, a microdialysis probe with a molecular mass cut-off of 20 000 (CMA 60; CMA/Microdialysis AB, Solna, Sweden) was inserted into one thigh muscle and into the subcutaneous adipose tissue at the ventro-lateral side of the thigh under aseptic conditions by use of a guidance cannula. The probe was constantly perfused with Ringer’s solution at a flow rate of 1.5 µL/min by means of a precision pump. After a 60 min equilibration period, 600 mg linezolid (Zyvoxid; Pharmacia, Erlangen, Germany) was administered intravenously to patients. Sampling of microdialysates and venous blood was performed at pre-defined time points. Prior to drug administration, the individual recovery values of linezolid were determined using the ‘retro-dialysis method’. For that reason, linezolid was added at a concentration of 10 mg/L to the perfusion fluid and its rate of disappearance through the microdialysis membrane was determined. The individual recovery was calculated by using the mean value of two measurements by the following equation: recovery (%) = 100 – (100 × C_dialysate/C_perfusate). Blood was collected in tubes containing the lithium salt of heparin, kept on ice for a maximum of 30 min, and centrifuged at 2550 g for 5 min at 4°C. Plasma and microdialysates were stored at approximately −70°C until analysis. Linezolid was quantified in the three matrices by a validated HPLC method.

The individual protein-binding values were used for the determination of free linezolid concentrations in plasma. Pharmacokinetic calculations were carried out using commercially available computer software (Kinetica, version 3.0; Innaphase, Philadelphia, PA, USA). Concentrations at 12 and 24 h were calculated by the following equation: C = C_F × e^(-k×t), where C is the concentration at 12 or 24 h, C_F the last concentration measured in vivo (at 8 h), k_el the elimination rate constant, and t the difference between C and the AUCs from 0 to 8 h (AUC_0–8) and 0 to 24 h (AUC_0–24) in plasma and interstitial fluid were calculated using the linear trapezoidal rule. For calculation of the total drug CL and the apparent volume of drug distribution at steady state (Vss), the oral dose of linezolid was not corrected for bioavailability (F) as this value is 1. CL and Vss of linezolid were calculated for plasma as follows: CL = dose × (F)/AUC_0–∞, where AUC_0–∞ represents the AUC from zero to infinity, and Vss = CL × MRT, respectively. MRT is the mean residence time. The AUC_0–24(bid) for 600 mg at steady state was corrected for twice-daily dosing by the equation AUC_0–24(bid) = AUC_0–12 × 2. Wilcoxon’s paired test was used for comparison of AUCs in plasma and interstitial fluids within individuals. A two-sided P value of less than 0.05 was considered significant.

**Results and discussion**

In total, 24 patients were enrolled into the study. The population consisted of 16 septic shock patients and 8 subjects suffering from severe sepsis. Healthy controls were derived exclusively from another study published previously by our study group. Sepsis was diagnosed and verified according to the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee. The mean plasma protein binding was 8.66 ± 3.14%, 15.14 ± 7.28% and 13.27 ± 6.38% in healthy subjects, septic patients and septic shock patients, respectively.

The mean concentration versus time profiles of linezolid in plasma and interstitium of soft tissues of healthy volunteers and septic patients are shown in Figure 1. After a single intravenous dose of 600 mg linezolid, the AUCs from 0 to 8 h and from 0 to 24 h in plasma and interstitium as well as peak concentrations of linezolid in plasma were essentially the same between septic cohorts (P > 0.05). The free fraction of linezolid in plasma equilibrated completely with the interstitial fluid of soft tissues as indicated by a ratio of almost 1 for fAUC_tissue to the fAUC of linezolid in plasma in all groups. However, a tendency to higher concentrations in subcutaneous adipose tissue was detected in healthy volunteers, i.e. a finding that was already observed in our previous study. In general, high inter-individual variability in the ability of linezolid to penetrate tissues was detected in severe septic and septic shock patients as indicated by a coefficient of variation ~50% (Table 1). Independent of the severity of sepsis, the very high inter-individual variability in the ability of linezolid to penetrate soft tissues rendered distinct septic patients to non-optimal linezolid concentrations in tissues resulting potentially in therapeutic failure of these patients. It would be of interest to know whether the lack of difference in tissue pharmacokinetics of linezolid between septic groups is due to the relatively low number of patients included in the study or due to a real similarity of both profiles. Given the sample size of 16 and 8 patients presenting with septic shock and severe sepsis, respectively, the power of this study was 80% to detect a difference of ~30% between groups. Smaller differences, however, were considered to be not clinically relevant after a single dose.

To calculate the clinical efficacy of linezolid, two PK–PD indices, i.e. the ratio of the fAUC_t to the MIC and the time above the MIC (t > MIC) are commonly used. However, most probably because of the moderately prolonged post-antibiotic effect of linezolid, the ratio of the fAUC_t to the MIC is considered the more relevant index for predicting the efficacy of linezolid therapy. In addition, it is a commonly accepted approach to estimate the steady-state concentrations of antibiotics based on the single-dose measurements. This approach, however, is useful in healthy volunteers, but offers a number of potential pitfalls in patients. Most importantly, this method may be used only under the assumption that the clinical presentation of patients remains stable over time, which means that no improvement or worsening
of the clinical condition occurs over the antibiotic treatment period. This is a precondition unlikely to be present in the clinical situation. More realistically, patients presenting with severe sepsis or septic shock need substantial replacement of the fluid to keep the mean arterial blood pressure sufficiently high for adequate organ perfusion. Changes in the total body water volume, caused by the administration of large volumes of fluids and resulting in tissue oedema, will particularly affect antibiotics that selectively distribute to the extracellular space fluid. In addition, vasopressors are commonly used in this setting. The volume of Table 1. Pharmacokinetic data of linezolid in plasma, muscle and subcutaneous adipose tissue after a single intravenous dose of 600 mg linezolid in healthy subjects and patients presenting with sepsis or septic shock

<table>
<thead>
<tr>
<th></th>
<th>AUC_{0–8} (mg.h/L)</th>
<th>AUC_{0–24} (mg.h/L)^a</th>
<th>AUC_{0–24} (mg.h/L)^b</th>
<th>C_{av(ss)} (mg/L)</th>
<th>C_{max} (mg/L)</th>
<th>T_{max} (h)</th>
<th>t_{1/2b} (h)</th>
<th>CL (L/h)</th>
<th>V_{ss} (L)</th>
</tr>
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<tbody>
<tr>
<td>Healthy (n = 10)</td>
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<tr>
<td>plasma free</td>
<td>52.98 ± 11.78</td>
<td>78.30 ± 24.97</td>
<td>159.84 ± 60.42</td>
<td>13.32 ± 5.03</td>
<td>12.84 ± 2.57</td>
<td>0.84 ± 0.17</td>
<td>4.73 ± 2.08</td>
<td>8.59 ± 3.38</td>
<td>51.47 ± 9.51</td>
</tr>
<tr>
<td>subcutis</td>
<td>77.43 ± 25.73</td>
<td>129.76 ± 46.18</td>
<td>272.87 ± 113.54</td>
<td>22.74 ± 9.46</td>
<td>18.72 ± 5.43</td>
<td>0.77 ± 0.26</td>
<td>5.72 ± 2.67</td>
<td>ND</td>
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<tr>
<td>muscle</td>
<td>60.28 ± 12.7</td>
<td>92.89 ± 23.33</td>
<td>194.46 ± 61.06</td>
<td>16.21 ± 5.09</td>
<td>13.18 ± 2.85</td>
<td>0.97 ± 0.18</td>
<td>5.39 ± 2.86</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Septic shock (n = 16)</td>
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<tr>
<td>plasma free</td>
<td>47.17 ± 13.59</td>
<td>70.78 ± 28.12</td>
<td>146.55 ± 66.54</td>
<td>12.21 ± 5.55</td>
<td>14.23 ± 3.45</td>
<td>0.52 ± 0.41</td>
<td>4.92 ± 2.08</td>
<td>9.81 ± 4.32</td>
<td>60.37 ± 13.92</td>
</tr>
<tr>
<td>subcutis</td>
<td>44.61 ± 19.38</td>
<td>65.76 ± 32.61</td>
<td>132.41 ± 68.64</td>
<td>11.03 ± 5.72</td>
<td>11.02 ± 4.80</td>
<td>0.86 ± 0.60</td>
<td>4.50 ± 1.82</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>muscle</td>
<td>42.76 ± 15.64</td>
<td>68.74 ± 32.84</td>
<td>146.31 ± 86.60</td>
<td>12.19 ± 7.22</td>
<td>9.33 ± 3.08</td>
<td>0.95 ± 0.68</td>
<td>5.41 ± 2.70</td>
<td>ND</td>
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<tr>
<td>Severe sepsis (n = 8)</td>
<td></td>
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<tr>
<td>plasma free</td>
<td>39.85 ± 14.49</td>
<td>50.91 ± 23.13</td>
<td>100.41 ± 46.68</td>
<td>8.37 ± 3.89</td>
<td>14.23 ± 4.13</td>
<td>0.49 ± 0.07</td>
<td>3.14 ± 1.53</td>
<td>14.83 ± 7.55</td>
<td>57.15 ± 17.8</td>
</tr>
<tr>
<td>subcutis</td>
<td>48.43 ± 22.80</td>
<td>67.70 ± 45.23</td>
<td>135.00 ± 96.97</td>
<td>11.25 ± 8.08</td>
<td>11.09 ± 4.35</td>
<td>1.67 ± 0.86</td>
<td>3.25 ± 1.70</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>muscle</td>
<td>40.04 ± 14.93</td>
<td>52.67 ± 26.00</td>
<td>101.95 ± 50.64</td>
<td>8.50 ± 4.22</td>
<td>10.12 ± 4.47</td>
<td>1.49 ± 1.24</td>
<td>2.99 ± 0.98</td>
<td>ND</td>
<td>ND</td>
</tr>
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</table>

AUC_{0–8}, AUC from 0 to 8 h; AUC_{0–24}, AUC from 0 to 24 h; t_{1/2b}, elimination half-life at the β-phase; C_{av(ss)}, average concentration at steady state calculated from single-dose measurements; C_{max}, maximum concentration; T_{max}, time to C_{max}; CL, apparent total body clearance; V_{ss}, apparent volume of distribution at steady state; ND, not determined.

Data are expressed as means ± SD.

Data on healthy volunteers derived from Dehghanyar et al.6

After single-dose administration.

Corrected for twice-daily dosage (τ of 12 h).
antibiotic distribution and drug CL will be affected directly by these measures, thereby confounding calculations on steady-state concentration if based on single-dose measurements.

Linezolid, however, is an antibiotic characterized by a volume of distribution ranging from 50 to 60 L (Table 1), providing circumstantial evidence that it distributes not exclusively to the extracellular space fluid, but also penetrates to certain extent into human cells. This makes linezolid less susceptible to extensive changes of the extracellular fluid volume, contrasting with very hydrophilic antibiotics such as fosfomycin and the class of β-lactams, which distribute predominantly into the extracellular space fluid and plasma compartment. Thus, the concentrations of β-lactam antibiotics, such as piperacillin and cefpirome, was shown to be several fold lower in tissues of septic shock patients when compared with patients presenting with sepsis or severe sepsis.4,10

Hence, on the basis of the single-dose measurements, we offer, in this study, a calculation of average linezolid concentrations in plasma and interstitium at steady state for a dosing regimen of 12 h. As expected from all the aspects discussed before, no major differences were detected between the pharmacokinetic profiles in plasma and interstitium derived from patients presenting with either severe sepsis or septic shock (P > 0.05). Thus, with minor limitations, the mean extracellular free concentrations of linezolid in soft tissues and plasma appeared to remain unaffected by the severity of sepsis. On the basis of the PK–PD calculations, linezolid concentrations should be sufficiently high to become effective in plasma and interstitium in most patients. However, a high inter-individual variability was observed between patients, potentially rendering individual subjects at increased risk of therapeutic failure.

In the present study, we provided evidence that linezolid penetrates to a high extent into the extracellular space fluid of septic patients. Its pharmacokinetic profile in plasma and tissues is comparable between patients presenting with either severe sepsis or septic shock. Therefore, in contrast to more hydrophilic antimicrobial agents such as fosfomycin and the class of β-lactam antibiotics, the pharmacokinetic profile of linezolid appeared not to be substantially affected by the severity of sepsis.

Acknowledgements

We are indebted to our study nurse, Petra Zeleny, for her essential contribution to this study.

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Transparency declarations

None to declare.

References