Biliary penetration and pharmacodynamic exposure of linezolid in liver transplant patients

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Objectives: The aim of the study was to assess the biliary penetration of linezolid and the probabilities of attaining optimal pharmacodynamic exposure against vancomycin-resistant enterococci (VRE) in the bile of liver transplant (LTx) patients who received linezolid for the treatment of multidrug-resistant Gram-positive hospital infections.

Methods: After at least 2 days of standard 600 mg twice-daily therapy, simultaneous bile and blood samples for linezolid assay were collected from six LTx patients just prior to drug administration to determine trough concentrations (Cmin) at steady-state in both sites. Linezolid concentrations in plasma and in bile were analysed by means of HPLC. Biliary penetration of linezolid was calculated as the ratio between Cmin in bile and in plasma. Optimal theoretical pharmacodynamic exposure of linezolid against VRE in bile was defined as biliary Cmin > MIC90.

Results: Cmin of linezolid in bile achieved very high values at steady-state, which were significantly higher than in plasma (median of 21.77 versus 8.08 mg/L, P = 0.021). The very high biliary penetration of linezolid (median value of 1.93; range 1.31–4.83) enabled achievement of optimal theoretical pharmacodynamic exposure against VRE in bile (Cmin > 2 mg/L) on all of the occasions.

Conclusions: These preliminary data suggest a potential role for linezolid in the treatment of cholangitis due to VRE in LTx patients. Obviously, further confirmatory data assessing also the AUC/MIC ratio of linezolid in bile are needed.

Keywords: bile, VRE, pharmacokinetics, trough concentration, cholangitis

Introduction

Linezolid is an oxazolidinone antibiotic active against most Gram-positive microorganisms whose role is especially valuable for difficult-to-access sites of infection and/or in the presence of multiresistant strains unresponsive to glycopeptides, including vancomycin-resistant enterococci (VRE).1

It was reported that Enterococcus faecalis and Enterococcus faecium are among the most frequently isolated pathogens in the bile of patients with acute cholangitis.2 Whereas E. faecalis is almost always susceptible to β-lactams and/or to glycopeptides, E. faecium resistant to both of these antibiotic classes is frequently detected as the causative microorganism in patients with hospital-acquired post-surgical infections, especially those who are immunocompromised or with indwelling stents.2 Of note, acquisition of VRE is a relatively frequent occurrence in solid organ transplant patients,3 especially in liver transplant (LTx) patients,4 in whom cholangitis was reported to be one of the most frequent infections.5 A recent Polish report showed a significant increase in the isolation of VRE from patients hospitalized in a transplant surgery ward over a 5 year period from 2001 to 2005 [from 0% of enterococcal isolates (0/400) in the 3 year period 2001–2003 to 18% (44/244) in 2005]. Interestingly, all of the VRE collected from different specimens

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were *E. faecium*, and half of the enterococcal strains isolated from bile in the last study year were VRE (10/20).6

It has been recently postulated that linezolid might represent a valuable choice for the treatment of cholangitis due to VRE,5 but to our knowledge no data on its biliary penetration exist. Indeed, the Tokyo guidelines for the treatment of acute cholangitis provide recommendations for consideration of biliary penetration of antimicrobials as one of the most important factors in drug selection.2

Previous studies in experimental animal models have shown that linezolid exhibits time-dependent antibacterial activity, so that given these pharmacodynamic characteristics one would predict that the duration of time serum concentrations exceed the MIC (t > MIC) would be the pharmacodynamic parameter that most strongly correlated with efficacy of linezolid.9 However, the AUC/MIC ratio was also shown to be important for its efficacy.9 Even in humans, t > MIC and AUC/MIC were shown to be highly co-related for linezolid efficacy.9 In a retrospective non-blinded analysis of 288 patients enrolled under the manufacturer’s compassionate use programme of linezolid, maximal efficacy in the treatment of bacteraemia, lower respiratory tract infection and skin and soft tissue infections was found in the presence of both persistent plasma trough concentration (Cmin) above the MIC (Cmin > MIC) and AUC/MIC values of 80–120.10 Accordingly, it may be hypothesized that maintenance of Cmin > MIC may represent an optimal pharmacodynamic determinant of efficacy for linezolid1 and that achievement of sufficiently high Cmin by also allowing high AUC, may be worthwhile.

The intent of our study was to assess the biliary penetration of linezolid and the probabilities of attaining optimal pharmacodynamic exposure against VRE, in terms of Cmin > MIC90, in the bile of LTx patients receiving linezolid for the treatment of multidrug-resistant Gram-positive hospital infections.

**Patients and methods**

All consecutive LTx patients in whom a T-tube was used for splinting the biliary anastomosis between the donors’ and recipients’ bile ducts and who received linezolid for the treatment of multidrug-resistant Gram-positive hospital infections during the period April 2004–April 2008 were included.

After at least 2 days of standard 600 mg twice-daily therapy, simultaneous bile and blood samples for linezolid assay were collected, from the T-tube and the cubital vein, respectively, just prior to drug administration to determine Cmin at steady-state in both sites. Whenever feasible, confirmatory samplings were investigated in the subsequent days.

Since this was an observational study of regular clinical practice, no ethics approval was necessary. Therapeutic drug monitoring of plasma Cmin is widely accepted as a valuable tool for the optimal management of time-dependent antibacterial agents in critically ill patients and is usually applied at our university hospital in routine clinical practice.

Linezolid concentrations in plasma and in bile were analysed by means of a validated HPLC analysis method, as described previously.11 Precision and accuracy were assessed by performing replicate analyses of quality control samples against calibration standards, intra- and inter-assay coefficients of variation always being <10%. The lower limit of detection was 0.2 mg/L.

Biliary penetration of linezolid was calculated as the ratio between Cmin in bile and in plasma in each single patient.

Optimal theoretical pharmacodynamic exposure of linezolid against VRE in bile was defined as biliary Cmin > 2 mg/L. The rationale behind this choice derives from the notions that Cmin > MIC may represent a valuable pharmacodynamic determinant of efficacy for the time-dependent antibacterial activity of linezolid1,10 and that the MIC90 of linezolid for VRE is 2 mg/L.12 The Kolmogorov–Smirnov test was performed to assess whether data were normally or non-normally distributed. Accordingly, descriptive data were expressed as mean ± SD or as median and range. Statistical analysis comparing data in bile and in plasma was performed using a parametric (paired Student’s t-test) and a non-parametric test (Mann–Whitney rank sum test) for normally and non-normally distributed data, respectively, by means of SigmaStat software (SPSS Science Software GmbH, Erkrath, Germany). A value of *P* < 0.05 denoted statistical significance.

**Results and discussion**

A total of six patients (five male and one female; 56.7 ± 6.1 years; 71.5 ± 8.5 kg) were included. Cmin of linezolid in bile achieved very high values at steady-state, which were significantly higher than in plasma (median of 21.77 versus 8.08 mg/L, *P* = 0.021) (Table 1 and Figure 1).

The very high biliary penetration of linezolid (median value of 1.93) may be explained by the fact that this drug may be a substrate of P-glycoprotein,13,14 the extrusive pump that is highly represented in the biliary pole of the hepatocyte. This means that, similarly to other drugs that behave as xenobiotics, a significant amount of drug may be excreted in the bile. Indeed, the high bile-to-plasma ratio observed for linezolid in our study may be affected by sampling only at one time point. Considering that drug concentration ratios may change over time (systemic hysteresis), this means that there may be a potential for variance of biliary penetration at different times after dosing.

These preliminary data suggest a potential role for linezolid in the treatment of cholangitis due to VRE in LTx patients. Optimal theoretical pharmacodynamic exposure against VRE in bile, in terms of Cmin > MIC90, was achieved on all of the occasions (Figure 1). Additionally, two of the major recommendations of the Tokyo guidelines for drug selection in the treatment of acute cholangitis, namely consideration for both in vitro susceptibility and good biliary penetration, were met.5 We

<table>
<thead>
<tr>
<th>Patient</th>
<th>Bile Cmin (mg/L)</th>
<th>Plasma Cmin (mg/L)</th>
<th>Bile/plasma ratio</th>
</tr>
</thead>
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<tr>
<td>1</td>
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<td>6.10</td>
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<tr>
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<td>8.54</td>
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</tr>
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<td>19.16</td>
<td>1.35</td>
</tr>
<tr>
<td>Range</td>
<td>7.23–51.82</td>
<td>2.07–27.87</td>
<td>1.31–4.83</td>
</tr>
</tbody>
</table>

**Table 1.** Trough concentrations (Cmin) in bile and in plasma and Cmin bile-to-plasma ratio of linezolid in six LTx patients
Biliary concentrations of linezolid in liver transplant patients

![Figure 1](image)

Figure 1. Box (median, and 25th and 75th percentiles) and whisker (5th and 95th percentiles) plots of trough bile and plasma concentrations of linezolid observed in six LTx patients treated with 600 mg twice daily. Optimal theoretical pharmacodynamic exposure of linezolid against VRE in bile was defined as biliary $C_{\text{min}} > 2 \text{ mg/L}$.

recognize that an assessment of the AUC would have been more informative, since it could have allowed a more exhaustive estimation of both the pharmacodynamic determinants of efficacy for linezolid in bile. However, maintenance of $C_{\text{min}} > \text{MIC}$ at the infection site is currently considered a relevant determinant of efficacy for the time-dependent antibacterial activity of linezolid.\(^{1,8,10}\) Additionally, considering the high biliary $C_{\text{min}}$ exceeding the concentration of 7 mg/L on all of the occasions, it may be reasonably supposed that AUC/MIC higher than 80 might be achieved in bile with a standard dosing regimen.

A wide inter-individual pharmacokinetic variability was observed, similarly to what has previously been observed either by ourselves or by other authors during treatment of critically ill patients with linezolid.\(^{11,14}\) The very high plasma $C_{\text{min}}$ of linezolid observed in two out of the six patients may have two possible explanations. First, it has been postulated that the non-linear pharmacokinetics of linezolid observed in some cases might be the result of an autoinhibition of the formation of the major linezolid metabolite due to the inhibition of respiratory chain enzyme activity.\(^{14}\) Interestingly, the presence of hyperlactacidemia (9.5 mmol/L) in one of these two patients, i.e. of a drug-related adverse event potentially occurring in the presence of linezolid overexposure and due to the same pathogenetic mechanism,\(^{13}\) seems to support this hypothesis. Additionally, linezolid overexposure may be due to the co-treatment with P-glycoprotein inhibitors.\(^{13}\) Of note, all of our LTx patients were co-treated with immunosuppressants that act as P-glycoprotein inhibitors (three with tacrolimus, two with everolimus and one with cyclosporin), and some also received other P-glycoprotein inhibitors (omeprazole in three cases).

We are well aware of the methodological limitations of our study, particularly the limited sample size, which is too small to draw definite conclusions, and the fact that the observational nature of the study obliged us to limit sampling to only one time point. This means that AUC needs to be elucidated to better define the pharmacodynamics of linezolid in the biliary tract.

In conclusion, these preliminary data of optimal theoretical pharmacodynamic exposure in bile suggest a potential role for linezolid in the treatment of cholangitis due to VRE in LTx patients. Obviously, further exhaustive confirmatory data are needed.

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Transparency declarations
F. Pea has received funds for speaking at symposia organized on behalf of Pfizer. P. V. has received funds for speaking at symposia organized on behalf of Pfizer and has also received funds for research from Pfizer. F. Pavan was a post-graduate fellow at the Institute of Clinical Pharmacology and Toxicology, University of Udine at the time of the study period, and is currently employed at GlaxoSmithKline, Verona, Italy. All other authors: none to declare.

References