Effect of Pyridoxine, Rifampin, and Renal Function on Hematological Adverse Events Induced by Linezolid: a Comparative Study.

Alex Soriano¹, Mar Ortega¹, Sebastián García², Georgina Peñarroja¹, Albert Bové³, Miguel Marcos¹, Juan C Martínez², José A Martínez¹ and Josep Mensa¹.

¹ Department of Infectious Diseases, Hospital Clínic of Barcelona. C/ Villarroel 170. Barcelona 08036, Spain.
² Department of Orthopedics and Traumatology, Hospital Clínic of Barcelona.
³ Department of Internal Medicine, Hospital Clínic of Barcelona.

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Correspondence:
Alex Soriano.
Department of Infectious Diseases. Hospital Clínic of Barcelona.
C/ Villarroel 170.
Barcelona 08036, Spain.
e-mail: asoriano@clinic.ub.es
Telephone: 00+34+932275708.
Fax: 00+34+934514438.
Abstract

Hematological disturbances that develop during linezolid treatment are a major concern when it is administered for prolonged periods of time. The aim of this study was to evaluate the influence of pyridoxine, rifampin, and renal function on hematological adverse events. From January 2002 to April 2006, 52 patients received a long-term course of linezolid. Blood cell counts were monitored weekly. Thrombocytopenia was defined as a decrease to <75% of baseline platelet count and anemia when the haemoglobin concentration decreased to ≥2 g/L from baseline value. 24 patients received linezolid alone and 28 linezolid plus 200 mg of pyridoxine. The Kaplan-Meier survival method followed by the log-rank test was used to estimate the cumulative probability of adverse events and the Cox regression analysis was performed to evaluate independent predictors of toxicity. Baseline characteristics of patients in both groups were similar. The cumulative probability of thrombocytopenia and anemia was not different in patients that received pyridoxine compared with those that did not receive it. Hematological adverse events were less frequent in patients taking rifampin and more frequent in patients with renal failure. However; the Cox regression analysis showed that rifampin was the only independent predictor associated with a lower risk of thrombocytopenia (HR:0.37, CI95%: 0.14-0.98, p=0.045). In conclusion, pyridoxine did not prevent linezolid-related hematological adverse events and the co-administration of rifampin was associated with a lower risk of thrombocytopenia.
Introduction

Linezolid belongs to a family of antimicrobials (oxazolidinones) that inhibit bacterial protein synthesis by preventing the fusion of 30S and 50S ribosomal subunits (14). Linezolid has shown an excellent efficacy against Gram-positive cocci including *Staphylococcus aureus*, coagulase-negative staphylococci, enterococci, and streptococci with a range of MIC from 0.5 to 4 \( \mu \)g/mL (12). Furthermore, linezolid has a 100% oral bioavailability and reaches high concentrations in different tissues (skin, synovial fluid, bone, cerebrospinal fluid, lung, and eye). Therefore, it seems to be a good alternative for the treatment of orthopaedic implant infections, ventricle-peritoneal shunts, and other infections related with foreign bodies where Gram-positive cocci are the main pathogens and where prolonged courses of antimicrobial therapy are needed. However, a major concern with this antibiotic is its safety profile, especially when it is administered for more than 4 weeks (10).

The most important adverse events are hematological disturbances, especially thrombocytopenia and anemia (1,4,11,17,19,22), but the underlying mechanisms to explain this toxicity are still unknown. Spellberg B et al described that the administration of pyridoxine (vitamin B6) was able to revert linezolid-related thrombocytopenia and anemia in 2 patients (24). Although there is no clear mechanism to explain this effect, it is reasonable to evaluate the potential preventive effect of pyridoxine on hematological disturbances in patients receiving prolonged courses of linezolid. In the present study, we evaluated the influence of pyridoxine on the frequency of hematological disturbances in 2 consecutive cohorts (with and without pyridoxine) with similar baseline characteristics and that received prolonged courses of linezolid as well.
as the influence of other clinically relevant variables such as renal function and
the co-administration of rifampin.
**Patients and methods**

From January 2002 to April 2006, patients who received a long-term course (≥3 weeks) of linezolid (Zyvoxoid; Pfizer) were identified and monitored in a tertiary University Hospital in Barcelona, Spain. The protocol included a weekly blood cell count, serum creatinine, and serum glucose level. From December 2004 to April 2006, oral administration of pyridoxine (Godabion, Merck Pharma Quimica) at a dosage of 200 mg once daily was added to linezolid treatment. A total of 52 patients were included in the protocol. In the first 24 patients, linezolid was administered alone and in the remaining 28, pyridoxine was added. The clinical variables gathered were, age, sex, type of infection, etiologic microorganism, co-morbidity, baseline serum creatinine, and estimated baseline glomerular filtrate (using the Cockcroft-Gault formula, GF (mL/min)=(140-age in years) x weight (Kg) / 72 (or 85 for women) x serum creatinine (mg/dL)), length of linezolid treatment (days), co-administration of rifampin, baseline level of platelet count (x10^9 platelets/L) and baseline level of haemoglobin concentration (g/L). Thrombocytopenia was defined as a decrease in platelet count to <75% of the baseline value and anemia when the haemoglobin concentration decreased to ≥2 g/L from baseline value and without other plausible explanation. No other medications with potential hematological toxicity were administered to any patient. The ethical committee of the hospital approved the administration of pyridoxin and the patients signed the informed consent.

**Statistical analysis**

Continuous variables were expressed as mean and standard deviation (SD) and median and interquartil range (IQR) and were compared using an unpaired Student’s t-test. Categorical variables were compared using Fisher’s exact test.
or Chi-square test when necessary. The cumulative probability of thrombocytopenia and anemia was estimated by univariate analysis using the Kaplan-Meier survival method followed by the log-rank test. Furthermore, a stepwise forward Cox regression analysis was performed to evaluate independent predictors of hematological toxicity. Variables with a p-value less than 0.15 in the univariate analysis were entered into the multivariate analysis, variables achieving p<0.05 in the final model were considered significant. Hazard ratio (HR) with 95% confidence interval (CI) was calculated for each significant variable. The statistical analysis was performed using the SPSS 12.0 package (SPSS, Chicago, IL).
Results

The baseline characteristics of patients are shown in table 1. There were no significant differences regarding baseline parameters between patients that received or did not receive pyridoxine.

The survival curves demonstrated that the cumulative probability of thrombocytopenia and anemia was not different in patients that received pyridoxine compared with those that did not receive it (figure 1a and b).

Linezolid was stopped due to severe thrombocytopenia (<100x10^9 platelets/L) in 7 patients out of 52 (13.4%), in 3 out of 24 patients (12.5%) that received linezolid alone and in 4 out of 28 (14.2%) that received pyridoxine (p=0.58).

Linezolid was stopped due to severe anemia (<8 g/L) in 4 out of 52 cases (7.7%), in 3 out of 24 patients (12.5%) that received linezolid alone and in 1 out of 28 (3.5%) that received pyridoxine (p=0.24).

Rifampin was added to linezolid in 17 patients with an orthopaedic implant infection in which the implant was not removed. Age, length of linezolid treatment, basal serum creatinine, glomerular filtrate, platelet count and haemoglobin concentration were not different between patients that received or did not receive rifampin. The cumulative probability of thrombocytopenia was significantly lower in patients that received rifampin than in those that did not receive it (p=0.02) (figure 2a). Only 1 out of 17 (5.8%) patients taking rifampin had severe thrombocytopenia (<100x10^9 platelets/L) compared with 6 out of 35 (17.1%) patients that did not receive rifampin. There was a trend towards a lower cumulative probability of anemia in the rifampin group but the difference did not reach statistical significance (p=0.08) (figure 2b). The cumulative probability of hematological adverse events was analyzed according to the
glomerular filtrate. The risk of thrombocytopenia was significantly higher when the basal glomerular filtrate was <50 mL/min (p=0.02) (figure 3a). There was a trend towards a higher cumulative probability of anemia when the basal glomerular filtrate was <50 mL/min, however, the difference was not statistically significant (p=0.14) (figure 3b). The Cox regression model showed that the co-administration of rifampin was the only factor independently associated with lower risk of thrombocytopenia (HR:0.37, CI95%: 0.14-0.98, p=0.045). No variable was independently associated with the development of anemia.
Discussion

The activity against a broad range of Gram-positive cocci, high oral bioavailability (100%), and the good results described in studies on bone infections (3,20,21) makes linezolid an attractive oral alternative to glycopeptides in infections that require prolonged antimicrobial therapy. However, thrombocytopenia and anemia are common adverse events when linezolid is administered for more than 3 weeks. The administration of 50 mg orally once a day of pyridoxine (vitamin B6) to 2 patients, who developed hematological disturbances, was useful in reverting these adverse events (24).

In a recent article, Plachouras et al (18) administered 125 mg of pyridoxine to 24 patients that received linezolid for bone infections and the rate of thrombocytopenia (<140x10^9 platelets/L) and anemia (hematocrite <30%) was 45.8% and 25%, respectively. These data suggest that pyridoxine does not prevent linezolid-related hematological adverse events since the frequency of hematological adverse events were similar or even higher than those reported without pyridoxine (19,22). This was a non-comparative study where variables such as age, sex, co-morbidity, co-administration of other antibiotics, or renal function that could influence the rate of cytopenias were not controlled. In our study, the influence of pyridoxine on hematological adverse events was analyzed comparing 2 consecutive cohorts with similar baseline characteristics and length of linezolid treatment. The cumulative probability of thrombocytopenia and anemia was similar in both groups; therefore, our findings support the lack of protective role of pyridoxine.

Rifampin is the most active antibiotic against biofilm forming microorganisms, but it should not be administered alone due to the high risk of selecting resistant
mutants (2,27). It is of note that the administration of rifampin was independently associated with a lower risk of thrombocytopenia. Although in vitro studies have demonstrated that linezolid is not metabolized by human cytochrome P450 (26), recently Egle et al (7) observed in 8 healthy men, a decrease of serum linezolid concentration down to 35% after the administration of 600 mg of rifampin. They hypothesized that linezolid may be a substrate of P-glycoprotein whose expression is rapidly induced by rifampin and, as a consequence, intestinal secretion of linezolid may be increased. On the other hand, hematological adverse events were more frequent in patients with renal failure as previously described by other authors (13,25). Although linezolid does not require dosage adjustment in renal failure, its area under the serum concentration curve (AUC) is higher in this situation than when the renal function is normal (5). These findings suggest that hematological adverse events could be related to linezolid serum concentration.

The underlying mechanism of hematological adverse events is unknown. Our group has recently described that linezolid inhibits the mitochondrial ribosomes (9,23) and perhaps, this effect may be the cause of hematological alterations. Since the efficacy of linezolid (inhibition of bacterial ribosomes) is associated with the degree of exposure of the microorganism to the antibiotic measured by the AUC/MIC ratio (6), it is reasonable to assume that inhibition of mitochondrial ribosome may also be associated with the degree of linezolid exposure. These hypotheses are supported by the relationship between the linezolid AUC and the development of thrombocytopenia (8).

McKee et al (16) reported that linezolid concentration that inhibits 50% of mitochondrial protein synthesis (IC_{50}) in rats and rabbits’ heart and liver
mitochondria is between 3.37 to 5.26 µg/mL. Using the standard dosage of 600 mg/12h, the trough serum concentration at a steady state is 6 µg/mL (15) and the expected AUC$_{24h}$ is 260 mg·L/h. When the MIC of the etiologic agent is 1 or 2 µg/mL, the AUC/MIC ratio obtained with the standard regimen is 260 or 130 mg·L/h, respectively. In both cases, the AUC/MIC ratio is > 50-80 that is the target associated with the highest linezolid efficacy against Gram-positive cocci (6). Therefore, in order to reduce the adverse events, in those cases that need a prolonged treatment with linezolid (>21 days) and when the MIC of the etiologic agent is ≤2 µg/mL, linezolid concentration could be reduced to obtain an AUC/MIC ratio of about 100.

The present study has 2 major drawbacks. Firstly, this was a non-randomised study, however; the principle baseline characteristics that could influence hematological adverse events such age, sex, co-morbidity, other antibiotics, baseline hematological parameters, and renal function in both cohorts were similar. Secondly, the low number of patients reduces the statistical power of the study.

In conclusion, our data showed that 200 mg of pyridoxin once daily does not prevent linezolid-related hematological adverse events. The co-administration of rifampin was associated with a lower rate of hematological adverse events while renal failure with a higher rate. Since these factors affect linezolid serum concentration, these findings suggest that hematological toxicity is directly related to the degree of linezolid exposure. In the future, it is necessary to evaluate if the adjustment of serum concentration to obtain the pharmacodynamic target would be a reasonable strategy to avoid adverse events in prolonged courses of linezolid.
Aknowledgements

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Potential conflict of interest: we declare no conflict of interest in connection with this article.
REFERENCES


Table 1. Characteristics of patients at baseline.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non B6 (n=24)</th>
<th>B6 (n=28)</th>
<th>p</th>
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<tr>
<td>Age</td>
<td>64.7 (19.2)</td>
<td>66 (18.2)</td>
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<td>Sex (% of males)</td>
<td>58.3</td>
<td>67.8</td>
<td>ns</td>
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<tr>
<td>Type of infection (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Prosthetic joint infection</td>
<td>18 (75)</td>
<td>17 (60.7)</td>
<td>ns</td>
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<td>Osteomyelitis</td>
<td>2 (8.3)</td>
<td>3 (10.7)</td>
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<tr>
<td>Others</td>
<td>4 (16.7)</td>
<td>8 (28.5)</td>
<td>ns</td>
</tr>
<tr>
<td>Pathogen (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MR S. epidermidis</td>
<td>15 (62.5)</td>
<td>14 (50)</td>
<td>ns</td>
</tr>
<tr>
<td>MR S. aureus</td>
<td>4 (16.7)</td>
<td>5 (17.8)</td>
<td>ns</td>
</tr>
<tr>
<td>Enterococcus sp</td>
<td>-</td>
<td>3 (10.7)</td>
<td>ns</td>
</tr>
<tr>
<td>Others</td>
<td>2 (8.3)</td>
<td>3 (10.7)</td>
<td>ns</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (12.5)</td>
<td>3 (10.7)</td>
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<tr>
<td>Underlying diseases (%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Diabetes Mellitus</td>
<td>3 (12.5)</td>
<td>6 (21.4)</td>
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<td>Rheumatoid arthritis</td>
<td>2 (8.3)</td>
<td>1 (3.5)</td>
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<tr>
<td>Chronic renal failure</td>
<td>-</td>
<td>2 (7.1)</td>
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<tr>
<td>Solid neoplasm</td>
<td>1 (4.1)</td>
<td>4 (14.2)</td>
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<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>1 (4.1)</td>
<td>-</td>
<td>ns</td>
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<tr>
<td>Mean (SD)/median (IQR) length of linezolid treatment, days</td>
<td>55.5 (28.4) / 50.8 (29.9) /</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Nº of patients with &gt;56 days on treatment with linezolid</td>
<td>7</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Co-administration of rifampin (%)</td>
<td>7 (29.1)</td>
<td>10 (35.7)</td>
<td>ns</td>
</tr>
<tr>
<td>Mean (SD)/median (IQR) serum creatinine (mg/dL)</td>
<td>1 (0-9-1.2)</td>
<td>0.95 (0.8-1.3)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)/median (IQR) Glomerular filtrate (mL/minute)*</td>
<td>70.4 (27.2) / 70.7 (31.8) /</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Nº (%) of patients with GF:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;50</td>
<td>19 (67.8)</td>
<td>17 (70.8)</td>
<td>ns</td>
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<tr>
<td>30-50</td>
<td>7 (25)</td>
<td>7 (29.2)</td>
<td></td>
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<tr>
<td>&lt;30</td>
<td>2 (7.2)</td>
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<tr>
<td>Platelets count (x10⁹ platelets/L)</td>
<td>303 (113)</td>
<td>331 (130)</td>
<td>ns</td>
</tr>
<tr>
<td>Hemoglobin count (g/dL)</td>
<td>11.4 (1.5)</td>
<td>11.1 (1.5)</td>
<td>ns</td>
</tr>
</tbody>
</table>
glomerular filtrate was estimated using the Cockroft-Gault formula (see text).
MR, methicillin-resistant.
Figure 1. Cumulative probability of hematological adverse events in patients that receive or did not receive pyridoxine.

Days on treatment with linezolid

Log Rank test, p=0.27

Log Rank test, p=0.36

a) thrombocytopenia

b) anemia
Figure 2. Cumulative probability of hematological adverse events in patients that receive did or did not receive rifampin.
Figure 3. Cumulative probability of hematological adverse events according to the glomerular filtrate.

- a) thrombocytopenia
- b) anemia

Log Rank test, p=0.02
Log Rank test, p=0.14