Short communication

Linezolid plasma concentrations and occurrence of drug-related haematological toxicity in patients with Gram-positive infections

Dario Cattaneo a,*, Giovanna Orlando b, Valeria Cozzi a, Laura Cordier b, Sara Baldelli a, Stefania Merli b, Serena Fucile a, Cecilia Gulisano b, Giuliano Rizzardini b, Emilio Clementi c,d

a Unit of Clinical Pharmacology, Luigi Sacco University Hospital, Via G.B. Grassi 74, 20157 Milan, Italy
b Infectious Disease Department, L. Sacco University Hospital, Milan, Italy

Abstract

Retrospective studies have documented a significant association between linezolid (LNZ) plasma concentrations and drug-related haematological toxicity. However, the safe upper threshold level for LNZ plasma trough concentrations (Cmin values) has not been defined with certainty. A prospective observational study was performed aimed at comparing LNZ Cmin values in patients developing drug-related side effects with those measured in patients not experiencing LNZ toxicity. LNZ Cmin values were measured from the first week after starting therapy and were repeated periodically up to the end of treatment. Fifty patients, for a total of 210 LNZ Cmin evaluations, were considered. All patients (n = 9) who developed drug-related haematological toxicity also had significantly higher plasma LNZ Cmin values during the first week of therapy (9.0 ± 6.4 mg/L vs. 4.9 ± 3.7 mg/L; P < 0.01) and thereafter (9.3 ± 5.4 mg/L vs. 4.4 ± 3.4 mg/L; P < 0.01). The significant association between LNZ plasma concentrations and haematological toxicity was also confirmed by multivariate logistic regression analysis including age, serum creatinine and concomitant medications as independent variables. A causal relationship between LNZ concentrations and the risk of developing drug-related haematological toxicity was observed. Accordingly, application of therapeutic drug monitoring may improve the safety outcome of patients receiving LNZ therapy.

© 2013 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.

1. Introduction

Linezolid (LNZ) is an oxazolidinone antibiotic characterised by a wide spectrum of activity against Gram-positive pathogens resistant to β-lactams and glycopeptides and whose use has been progressively increased in recent years [1,2]. Clinical trials have shown that LNZ is safe and generally well tolerated. The major adverse event associated with LNZ treatment is reversible myelosuppression, mostly thrombocytopenia (and, to a lesser extent, leukopenia and anaemia), eventually requiring drug discontinuation [1–3].

Elevated LNZ plasma trough concentrations (Cmin values) have been reported in patients with renal or hepatic dysfunction who experienced thrombocytopenia after starting treatment with LNZ [4–6]. Similarly, 4–5-fold increases in LNZ peak serum concentrations and area under the serum concentration–time curve have been reported in critically ill patients compared with values measured in healthy volunteers [7].

These findings, together with the results of recent retrospective observations from single-centre experiences [8,9], suggest a potential relationship between the pharmacokinetics of LNZ and its tolerability, providing the rationale for targeting LNZ dosage to each individual patient based on therapeutic drug monitoring (TDM) of LNZ plasma concentrations. However, the safe upper threshold level for the LNZ plasma Cmin has not yet been conclusively defined [8,9].

The present prospective study was designed: (i) to evaluate the distribution of LNZ Cmin values in a routine clinical setting; (ii) to compare LNZ Cmin values in patients developing drug-related haematological toxicity with those measured in patients not experiencing clinical signs of LNZ toxicity; and (iii) to assess whether early measurement of plasma LNZ concentrations (i.e. in the first week after starting treatment) predicts subsequent development of drug-related side effects.

* Corresponding author. Tel.: +39 02 503 19643; fax: +39 02 503 19646. E-mail address: cattaneo.dario@hsacco.it (D. Cattaneo).

0264-8225/$ – see front matter © 2013 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.
http://dx.doi.org/10.1016/j.ijantimicag.2013.02.020
2. Patients and methods

2.1. Study population

Male and female patients referred to the Department of Infectious Diseases of Luigi Sacco University Hospital (Milan, Italy) requiring treatment with LNZ for any reason were enrolled in the present study. Paediatric subjects, patients with severe renal or hepatic impairment, and patients from intensive care units were excluded from the present study. A pathological blood cell count at baseline (before starting LNZ treatment) was an additional exclusion criterion.

All patients started LNZ treatment at 600 mg every 12 h given orally. LNZ dose adjustments were allowed throughout the study when deemed clinically appropriate (e.g. poor drug tolerability) and not according to TDM.

2.2. Study design

This was a prospective observational study carried out between February 2010 and June 2012 at L. Sacco University Hospital. The study was approved by the local Ethics Committee in November 2009. The study was aimed primarily at comparing LNZ C\textsubscript{min} values in patients who did or did not experience drug-related haematological toxicity during LNZ treatment.

From Day 3 after starting therapy with LNZ, eligible patients underwent evaluation of renal (as serum creatinine), hepatic (as serum transaminases) and haemorheological (haemochrome with formula) status, together with an assessment of drug C\textsubscript{min} values. These evaluations were repeated periodically (ideally at least once a week or in concomitance with the development of an adverse event) up to the end of LNZ therapy. Any relevant information on the clinical status of the patient was also recorded. The glomerular filtration rate (eGFR) was estimated using the Cockcroft–Gault formula as follows: eGFR = [140 − age] \times \text{body weight} / [\text{serum creatinine} \times 72]; in female patients, the formula was multiplied by a constant of 0.85.

The safety outcome was composed by episodes of anaemia (defined by a red blood cell count <3.0 × 10\textsuperscript{6}/μL), leucopenia (defined by a white blood cell count <2.5 × 10\textsuperscript{3}/μL) and/or thrombocytopenia (defined by a platelet count <125 × 10\textsuperscript{9}/μL).

2.3. Pharmacokinetic evaluations

Blood samples drawn into ethylene diamine tetra-acetic acid (EDTA)-containing Vacutainers\textsuperscript{®} (BD, Franklin Lakes, NJ) were collected from all patients 12 h after the last drug intake (a time window of ±5 min was considered acceptable), immediately before the next morning LNZ administration (trough concentrations). All samples were centrifuged at 3000 \times g and plasma was separated and stored at −20°C.

Plasma LNZ concentrations were determined using a validated high-performance liquid chromatographic (HPLC) method [10]. Briefly, after precipitation of plasma proteins with perchloric acid, the protein-free supernatant was separated by isocratic reverse-phase chromatography on an XBridge C18 column (Waters, Milan, Italy). The mobile phase consisted of a mixture of phosphoric acid 0.05%;acetonitrile (75:25, v/v) with a flow rate of 1 mL/min. The column eluate was monitored at 254 nm. The method was linear from 0.2 mg/L to 48 mg/L. The observed intraday and interday assay imprecision and accuracy were <10%.

2.4. Statistical analyses

Results are given as the mean ± standard deviation. Comparison of pharmacokinetic parameters between patients experiencing or not LNZ-related side effects was performed using the unpaired t-test. The ability of plasma LNZ concentrations to independently predict haematological toxicity was assessed by means of multivariate logistic regression analysis (MedCalc Software for Statistics in Medicine; MedCalc Software, Mariakerke, Belgium). The model included LNZ plasma C\textsubscript{min}, age, weight, serum creatinine and co-medications known to affect blood cell count as independent variables, and haematological toxicity (yes or no) as a dichotomous dependent variable. A P-value of <0.05 was considered statistically significant.

3. Results

3.1. Patient characteristics

Fifty patients were included in the present study. The main reasons for LNZ use were bone infections (30%), joint infections (25%) and drug-resistant tuberculosis (38%).

Of the 50 patients, 9 developed haematological toxicity. According to the World Health Organization (WHO) toxicity grading scale, four patients developed thrombocytopenia (all episodes were scored as grade 1), three patients developed anaemia (one episode was scored as grade 1 and two episodes were scored as grade 2), one patient developed leucopenia (scored as grade 2) and one patient developed pancytopenia (scored as grade 2). Such episodes appeared after a median of 12 days of LNZ treatment. All episodes resolved after LNZ withdrawal (n = 4) or drug dose reduction (n = 5). As shown in Table 1, patients experiencing haematological toxicity were significantly older and had worse renal function (i.e. higher serum creatinine concentrations and lower eGFR) compared with patients with good tolerability to LNZ treatment. No major differences were observed in the number of concomitant treatments between patients who did or did not develop haematological toxicity after starting treatment with LNZ (Table 1). None of the patients included in the present study were given rifampicin as co-medications.

3.2. Distribution of linezolid plasma trough concentrations

A total of 210 LNZ C\textsubscript{min} evaluations were performed. Overall, a wide distribution in the measured LNZ C\textsubscript{min} values was observed, with values ranging from 0.4 mg/L to 23.6 mg/L (Fig. 1). This distribution was associated with an interpatient variability in plasma LNZ concentrations of 63.7%. Intrapatient variability, expressed as the coefficient of variation measured in patients with at least three evaluations of LNZ plasma concentrations who did not change drug dose, was 30.6%.

3.3. Association between linezolid concentrations and haematological toxicity

First, all LNZ C\textsubscript{min} evaluations were pooled together and then divided in two groups, namely patients with or without haematological toxicity. Using this approach, patients experiencing haematological toxicity were found to have significantly higher LNZ C\textsubscript{min} values compared with patients with no clinical evidence of drug toxicity (9.3 ± 5.4 mg/L vs. 4.4 ± 3.4 mg/L; P < 0.01).

Subsequently, the analysis was repeated by considering only the first available LNZ plasma concentration measured in each patient, before the development of haematological toxicity. Using this approach, the 9 patients who subsequently developed drug-related haematological toxicity were found to have significantly higher plasma LNZ concentrations already during the first week of therapy, even before the development of the event (9.0 ± 6.4 mg/L).
vs. 4.9 ± 3.7 mg/L; \( P < 0.01 \)). This trend was also confirmed thereafter (Table 2). Noteworthy, patients having their LNZ dose reduced after the development of haematological toxicity reached, with the new LNZ dose, plasma drug concentrations comparable with those measured at the same time in patients not experiencing LNZ toxicity (Table 2).

As shown in Table 1, patients experiencing haematological toxicity were older and had higher serum creatinine concentrations compared with patients with good tolerability to LNZ treatment. To take into account these potential confounding factors, a multivariate logistic regression analysis was performed including age, serum creatinine, LNZ \( C_{\text{min}} \) and concomitant medications as independent variables and haematological toxicity as a dichotomous dependent variable. Also using this approach it was found that LNZ \( C_{\text{min}} \) values were significant predictors of haematological toxicity (\( P = 0.003 \)).

### 4. Discussion

This study identifies parameters for LNZ treatment useful for routine clinical practice. Wide interindividual variability in LNZ \( C_{\text{min}} \) values was found in patients treated with conventional LNZ doses. Taking advantage of multiple samples collected in the same patients during different visits, however, we were able to document that despite the wide interpatient distribution of LNZ \( C_{\text{min}} \) values, intrapatient variability was low (ca. 30%), which is a mandatory prerequisite for the feasible application of TDM.

A significant relationship between plasma LNZ \( C_{\text{min}} \) values and the risk of developing drug-related haematological toxicity was also observed. In particular, the plasma LNZ concentrations measured in patients who developed drug-related adverse events were approximately double compared with values measured in patients with no haematological toxicity. This trend was confirmed during each weekly assessment, also evidencing that the variability in LNZ \( C_{\text{min}} \) values within the two groups of patients (toxicity vs. no toxicity) was low.

A key finding emerging from this study is the time at which to perform TDM during LNZ treatment. Noteworthy, patients who
The freely available level, specifically designed in a recent study evaluating the non-linearity in the pharmacokinetics of LNZ and suggesting that the steady state could take even longer than 1–2 days to develop [12]. In the current study, the concentrations of LNZ measured at Day 3 are comparable with those collected thereafter, suggesting that this time is sufficient to reach a condition of steady state.

This approach may allow the early identification of patients at high risk of developing LNZ-related haematological toxicity before the actual onset of toxicity. Such an approach would allow a rational reduction in the daily drug dose thus improving LNZ safety and tolerability. This study was not intervention-based and most patients received fixed standard LNZ dosages not prospectively guided by TDM. Further investigations (such as a prospective comparison between fixed LNZ dose versus concentration-controlled in a randomised controlled clinical trial) will be required to confirm conclusively the relevance that TDM-guided dosage adjustments may have in preventing haematological toxicity.

An essential requirement for the use of TDM as a safety tool is the availability of clearly defined upper thresholds of the drug concentration associated with optimum drug response and limited drug-related toxicity. To date, however, such threshold levels for LNZ $C_{\text{min}}$ have not been defined with certainty. According to early observations in patients with renal insufficiency, LNZ concentrations of $>22\,\mu\text{g/mL}$ had been proposed in Japanese patients as a threshold level associated with higher risk of developing drug-related toxicity [4,5,13]. However, this threshold level has been challenged by the findings of a recent retrospective study involving adult patients on long-term LNZ treatment undergoing TDM [8]. In the latter study, by logistic regression analysis the authors found that the risk of drug-related thrombocytopenia was associated with LNZ $C_{\text{min}} > 8–10 \mu\text{g/mL}$ [8]. In the current prospective study specifically designed to assess whether TDM of plasma LNZ concentrations may help to improve the tolerability of this drug, it was documented that patients with haematological toxicity had mean LNZ $C_{\text{min}}$ values of ca. 9–10 $\mu\text{g/mL}$. Therefore, the results are in line with the study by Di Paolo et al. [14] and indicate that the upper threshold LNZ concentration of 22 $\mu\text{g/mL}$ resulting from the study in Japanese patients [6] is too permissive and should be lowered to ca. 10 $\mu\text{g/mL}$. The current findings, in agreement with previous observations [8,9,13,15], also suggest a potential prognostic role of TDM in the routine clinical management of adult patients treated with LNZ. Such an approach would be particularly useful in patients requiring prolonged LNZ therapy.

The present study was not designed for efficacy evaluation, and patients enrolled were given LNZ for any reason, irrespective of the type of infection. Thus, we cannot draw conclusions about the role of TDM as a potential tool to improve LNZ efficacy. However, considering that (a) for most pathogens the minimum inhibitory concentration (MIC) of LNZ is known [1,2,14] and (b) patients treated with the standard daily LNZ dose (600 mg twice daily) presented a wide distribution in LNZ $C_{\text{min}}$ values, it is reasonable to assume that stringent application of TDM may also help the early identification of patients underexposed to LNZ, having minimum drug concentrations below the MIC. In these patients, adjustment of drug dose and/or frequency of administration guided by TDM would eventually contribute to improve the response to LNZ therapy.

In conclusion, a significant association between high LNZ $C_{\text{min}}$ values measured from the first week of therapy and the risk of drug-related haematological toxicity was reported. Accordingly, early application of TDM may improve the safety outcome of patients on LNZ therapy.

Funding: No funding sources. Competing interests: None declared.

Ethical approval: This study was approved by the local Ethics Committee of Luigi Sacco University Hospital (Milan, Italy) in November 2009.

References