Disposition of linezolid in the isolated rat lung after systemic and pulmonary drug delivery

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Objectives: To characterize the distribution of linezolid in lung when it accesses this organ from the systemic circulation and when administered through the pulmonary route and to evaluate the influence of the ‘respiratory mode’ in the pulmonary distribution for both routes.

Methods: The study was conducted with 24 Wistar rats divided into four groups treated with linezolid under different experimental conditions. After the animals had been subjected to a tracheotomy followed by mechanical ventilation, the lungs were isolated. After a 5 min stabilization period, the antibiotic was administered through the systemic or the pulmonary route and samples of efferent fluid (EF) were collected using a previously programmed fraction collector. Samples of bronchoalveolar fluid (BALF) and of lung tissue were also taken at the end of each experiment. The concentrations of linezolid in the samples were determined using an HPLC technique with UV detection.

Results: The administration of linezolid through the inhalatory route significantly increased the levels of the drug in lung tissue and BALF with lung tissue/EF partition coefficients of 8.33 ± 2.51 as compared with 1.90 ± 0.78 for systemic administration. Also, the decrease in respiratory rate together with the increase in tidal volume favoured the process of linezolid distribution in pulmonary tissues and fluids.

Conclusions: Administration through the pulmonary route affords and excellent method for passively vectoring linezolid to the pulmonary fluids and tissues and the respiratory mode seems to affect the disposition of the antibiotic in this tissue for both administration routes.

Keywords: pulmonary distribution, pulmonary delivery, respiratory patterns

Introduction

Linezolid is an antibacterial agent belonging to the oxazolidinone group that acts by inhibiting the initiation of bacterial protein synthesis. It has a broad spectrum of activity against Gram-positive bacteria. Its efficacy has mainly been studied in endocarditis, hospital-acquired pneumonia (caused by Streptococcus pneumoniae and Staphylococcus aureus) and infections due to Gram-positive bacteria that are multiresistant to other antimicrobial agents, such as methicillin, penicillin and vancomycin. Also its use is recommended in skin and soft tissues infections.1

Of interest is the indication of linezolid for the treatment of pneumonia associated with mechanical ventilation and toxic shock syndrome.2 Some studies have shown that vancomycin may not be the best option for the treatment of pneumonia associated with mechanical ventilation caused by methicillin-resistant S. aureus, and that other antibiotics, such as linezolid and clindamycin, could afford more promising results for such infections. Kollef et al.,3 obtained results suggesting better treatment efficiency with linezolid in patients associated with mechanical ventilation than treatment with vancomycin at the same dose, particularly in cases with pneumonia due to methicillin-resistant S. aureus (62.2% versus 21.2%).

Although linezolid has been considered a bacteriostatic agent against enterococci or staphylococci and bactericidal against streptococci,4 in vivo assays carried out in animal models with severe infections caused by enterococci or staphylococci have caused doubt about the assumed bacteriostatic nature of the drug against these microorganisms.5 Regarding the pharmacokinetic characteristics of the drug, linezolid has an oral bioavailability of 95% to 100%, the maximum concentration being reached at 1–2 h, and a half-life of 5 h, with a low degree of plasma protein-binding (31%) and independent of the concentration.6 Its distribution volume is 0.5–0.8 L/kg, with access to most organs.
and tissues. With respect to its ability to access the respiratory system, there are clear discrepancies in the literature. Studies carried out in animals have reported a moderate capacity to access bronchial secretions, with levels in bronchoalveolar fluid (BALF) of $2.3 \pm 1.9$ mg/L against $12.7 \pm 2.9$ mg/L in plasma at 30 min after oral administration of the antibiotic (BALF concentration/plasma ratio $= 0.2$). In contrast, studies performed in humans suggest a greater affinity of linezolid for pulmonary fluids. In particular, in a study carried out on 25 healthy volunteers who received 600 mg of linezolid orally in multiple doses, the authors found mean concentrations in the fluid of the epithelial lining (ELF) of $64.3 \pm 33.1$ and $24.3 \pm 13.3$ mg/L, at 4 and 12 h, respectively, as compared with values of $15.5 \pm 4.9$ and $10.2 \pm 2.3$ mg/L in plasma at the same times. In alveolar cells, however, the mean linezolid concentrations were $2.2 \pm 0.6$ and $1.4 \pm 1.3$ mg/L at 4 and 12 h, respectively. These results seem to be hard to interpret because, on one hand, an ELF concentration/plasma ratio of 4.1 and 2.4 is obtained, which implies an excellent degree of transport of linezolid through the alveolar–epithelial barrier and, on the other, an alveolar cell/plasma ratio of 0.15 and 0.14 which, in contrast, demonstrates a low capacity for passage by the antibiotic through membranes. Other studies in humans have detected intermediate values in the ELF, as is the case of Honeybourne et al. who carried out work to determine and compare linezolid concentrations in serum, bronchial mucosa, ELF and macrophages at different time intervals after the administration of 600 mg of linezolid orally, finding values of $13.4$, $10.7$ and $25.1$ mg/kg in serum, bronchial mucosa and ELF, respectively, corresponding to a concentration ratio of 0.6 for mucosa/serum and 1.8 for ELF/serum. In critically ill patients with hospital-acquired pneumonia undergoing mechanical ventilation, who often have physiopathological conditions that may alter the pharmacokinetics of this agent, few studies of this type have been carried out. Boselli et al. performed a study in such patients and obtained mean concentrations of $17.7 \pm 4.0$ and $14.4 \pm 5.6$ (1 h post-infusion) and $2.4 \pm 1.2$ mg/L and $2.6 \pm 1.7$ mg/L (12 h post-infusion) in plasma and ELF, respectively; i.e. an ELF/plasma ratio of $\approx 1$ for both sampling times. Similar data were reported by Saralaya et al. in patients with cystic fibrosis, in whom the sputum concentration/serum ratio was $1.4 \pm 0.6$ at 2 h after oral administration of 600 mg of linezolid.

Regarding the safety of this antibiotic, it has been observed that the drug is able to elicit myelosuppression, in particular thrombocytopenia, in a dose-dependent manner. In a study of 686 critically ill patients treated with linezolid over 7–21 days at a dose of 600 mg/12 h, the authors found that the appearance of these adverse effects had a frequency (6.4% to 7.7%) similar to that described for vancomycin, and that this seems to be increased by prior treatment with the aminoglycopeptide. A recent study carried out by Bishop et al. detected a high proportion of adverse reactions, especially gastrointestinal and haematological toxicity. Again the appearance of side effects seemed to depend on the duration of treatment and they were more frequent in patients treated with linezolid for more than 14 days. All these data about the efficiency and safety indicate that linezolid could be an effective and safe drug, although its potential association with certain adverse reactions counsels the optimization of dosage regimens in order to avoid prolonged treatments. In the particular case of lung infections, with or without assisted ventilation, administration of the drug by inhalation may offer a way to optimize therapy with linezolid by simple passive vectoring to the site of infection, since the bibliographic data on the access to lung structures and fluids are discrepant and in some of the cases mentioned above only a moderate degree of access to those sites was seen. Success of antibiotic inhalation has been proved with tobramycin in cystic fibrosis patients receiving the aminoglycopeptide over 2 years.

The aim of this study was to characterize and compare the kinetics of linezolid in the pulmonary system after its administration through the respiratory and systemic routes and to assess the influence of the respiratory mode in the pulmonary disposition for both administration routes, using an animal model of artificially perfused and mechanically ventilated isolated lung.

Materials and methods

Experimental protocol

The study was performed using 24 Wistar male rats Crl:WI (Han) (Animal Experimental Service, University of Salamanca, Spain) with a mean body weight of 244.87 $\pm$ 8.27 g. Twelve hours prior to the experiments, the animals were isolated in cages and allowed access to tap water ad libitum. The housing and experimental treatment of animals were in accordance with the current Spanish legislation and comply with the ‘Principles of Laboratory Animal Care’. The study was approved by the Ethics Committee of Salamanca University for use of laboratory animals.

The animals were randomly distributed into the following groups of six rats each: (i) systemic drug administration, 60 respirations per minute (rspm) and 2 mL of tidal volume; (ii) systemic drug administration, 30 rspm and 4 mL of tidal volume; (iii) inhalation drug administration, 60 rspm and 2 mL of tidal volume; and (iv) inhalation drug administration, 30 rspm and 4 mL of tidal volume.

Isolated lung model

The method used to isolate the lungs and to keep them artificially perfused and mechanically ventilated has been described in depth previously. Briefly, the procedure is as follows: after weight recording, the animals were anaesthetized with sodium thiopental (Tiobaritual Braun $\approx 0.5$ g) (80 mg/kg, intraperitoneal route) and 1000 IU of sodium heparin (Heparina Rovi $\approx 5$%) was injected through the same route to avoid clotting. Then, tracheotomy and tracheal cannulation were performed with the animals in the decubitus supino position after which the cannula was immediately connected to a mechanical ventilation system (Respirator TSE ‘Advanced’ Model) (Technical and Scientific Equipment GmbH, Bad Homburg/Germany) set at 60 or 30 rspm and 2 or 4 mL of tidal volume. The ventilation system works under positive pressure and provides warmed and moistened air to the lungs. Next, the thorax was opened by two lateral transversal and one central longitudinal incision to expose the thoracic viscera. Insertion of a previously heparinized outflow cannula (left ventricle) and fixation of a heparinized inflow cannula (pulmonary artery) from the right ventricle were performed. The inflow cannula was connected to the mechanical perfusion pump (Minipuls, 3 Gilson) (Gilson International, Bad Camberg/Germany), which provided a non-pulsatile flow rate of 5 mL/min of the perfusion medium: Krebs–Henseleit bicarbonate (pH 7.4) (NaCl, KCl, CaCl₂, KH₂PO₄, MgSO₄, 7H₂O, NaHCO₃, Panreac, Spain) with glucose (0.9 g/L) (Panreac) and BSA fraction V (30 g/L) (pH 7.4) 1% 0.15 M NaCl, $\geq$98% agarose gel
electrophoresis, lyophilized powder). A probe (Transonic Systems, Inc. T106) was connected to the inflow cannula to measure the flow rate and a pressure transducer (Transpac® IV; Abbott Critical Care, Ithaca, NY, USA) was fitted to determine and record hydrostatic pressure at arterial level.

**Drug delivery**

Five minutes after starting the artificial perfusion (stabilization period), a dose of 400 µg of linezolid (Pfizer, 03KTH) was administered through the systemic or inhalation route.

**Systemic administration.** Four hundred micrograms of linezolid dissolved in 200 µL of perfusion medium was administered through the Y-device of the inflow cannula as a bolus injection.

**Pulmonary administration.** Four hundred micrograms of drug dissolved in 5 mL of distilled water was administered by inhalation. Nebulization of the drug solution was performed with aid of a nebulizer (Ultrasonic Aerosol Generator 700700-UV TSE system, Bad Homburg, Germany) connected to the artificial ventilation system in such a way that the nebulized product would reach the lungs through the cannula for 20 min.

**Sample collection**

Sample collection of efferent fluid (EF) was started at the same time of drug administration using a fraction collector (Gilson FC 203B Fraction Collector, Gilson Inc., Middleton, WI, USA) connected to the outflow cannula and programmed at the following time schedule for the systemic injection: 3 s intervals for the first min and 6 s intervals over the next min. Subsequently, sampling time intervals were 10 s for the next 2 min; 20 s for the next 2 min; 30 s for the next 2 min and 60 s for the last 2 min (total sampling period, 10 min; sample number, 54). For inhalation delivery, the same time schedule was applied for the first 10 min and then at 60 s intervals during the last 10 min (total sampling period, 20 min; sample number, 64).

After the EF sampling period, the tracheal cannula was disconnected from the ventilator and BAL was carried out using 0.3 mL of 0.9% saline solution. Then, the lung tissue was excised, weighed and a 1 g sample was separated for processing and used for the measurement of linezolid tissue concentrations.

**Quantification of linezolid**

Determination of linezolid concentrations in EF, BALF and lung tissue samples was carried out using an HPLC technique implemented in our laboratory from the method previously described. The mobile phase consisted of pH 7, 0.025 M phosphate buffer/acetonitrile (78:22). A Lichrocart® 55-4 Purospher®Star RP-18e (3 µm) column (Merck KGaA, Germany) was used as stationary phase. The column temperature was maintained at 28°C. Flow rate was 1 mL/min and the retention time of linezolid was 2 min. The Shimadzu (SCL-10Axl) liquid chromatographic system (Shimadzu Europa GmbH, Germany) was equipped with a model LC-10AD pump, a Waters 486 ultraviolet detector with wavelength λ = 250 nm and a Kontron oven controller (model 480). For data processing, the Class VP Data System (Shimadzu) was applied.

EF and BALF samples were assayed against calibration curves of linezolid prepared in Krebs–Henseleit medium. Owing to the wide range of drug concentrations in EF samples showing non-linear relationship for the whole range, three standard curves were prepared for linezolid quantification in EF: standard 1 for the lowest levels, 0.25–10 mg/L; standard 2 for the intermediate levels, 10–40 mg/L; and standard 3 for the highest levels, 40–200 mg/L. Standard, EF and BALF samples were processed as follows: 100 µL was mixed with 50 µL of 20% trichloroacetic acid (Panreac) and the mixture was vortexed for 30 s and then centrifuged (Microcentrifuge Abbott Laboratories, Germany) at 10 900 rpm to remove proteins. Fifty microlitres of supernatant was injected into the HPLC system.

**Pharmacokinetic analysis**

Linezolid concentration curves in the lung EF were analysed using statistical moment theory. According to this theory, the area under the concentration–time curve (AUC_{0→∞}) and the mean transit time (MTT) of linezolid in lung may be estimated from the stochastic analysis of the outflow concentration curve (C_t) according to the following equations:

\[
AUC_{0→∞} = \int_{0}^{∞} C(t)dt
\]

\[
MTT = \frac{\int_{0}^{∞} t \cdot C(t)dt}{\int_{0}^{∞} C(t)dt}
\]

Since the experimental system used here included tubing besides the isolated tissue, it was necessary to correct for the influence of these devices in the MTT estimated from the above equation. Additional experiments performed under the same experimental conditions as described above, but in absence of tissue, were carried out to quantify the mean transit time of the drug in the devices (MTT_d) in order to estimate the actual mean transit time of the drug in the tissue (MTT_t) by applying the following correction:

\[
MTT_t = MTT - MTT_d
\]

Assuming the experimental preparation to be a stationary system, the distribution volume of the drug in the lung (V) was calculated from MTT_t and the perfusion flow rate (Q = 5 mL/min), as follows:

\[
V = MTT_t \times Q
\]

The distribution coefficient (V/L_w) was also determined, L_w being the weight of the isolated lung. Finally, the washout rate constant (K_w) was estimated from the slope value of the terminal phase of the EF curve.

Corresponding partition coefficients were estimated from simultaneous concentrations in the three types of samples (EF, BALF and lung tissue).

**Statistical comparison**

The non-parametric Kruskal–Wallis test was used to compare results and the standard P < 0.05 value was considered for statistical significance.
Results

Figures 1 and 2 show the mean concentration curves of linezolid in EF after systemic and inhalatory administration for both respiratory patterns assayed. The EF profiles were significantly modified when tidal volume and respiratory frequency were changed for both administration modes. Table 1 shows the corresponding statistical moments together with derived pharmacokinetic parameter values of linezolid in isolated lung when the drug reached the pulmonary system from the systemic circulation. When administration was carried out through the inhalation route, the corresponding kinetic parameters were not estimated owing to the extremely low levels reached in EF, in particular those for the 2 mL of tidal volume and 60 rspm, which were below the quantification limit of the technique.

Comparison of systemic and pulmonary administration of the same dose of linezolid revealed a reduced access of the drug to the systemic space when administered by inhalation, regardless of the respiratory pattern, with a maximum level of 0.40 mg/L reached in EF for a nebulized dissolution at a concentration of 80 mg/L. In contrast, as shown in Figures 3 and 4, pulmonary delivery led to high levels in BALF (3.39 ± 1.50 and 9.55 ± 3.87 mg/L for 60 rspm; 2 mL of tidal volume and 30 rspm; 4 mL of tidal volume, respectively) as well as in lung tissue (1.55 ± 0.50 and 3.30 ± 0.91 μg/g for 60 rspm; 2 mL of tidal volume and 30 rspm; 4 mL of tidal volume, respectively), whereas systemic administration of the same dose led to undetectable concentrations in BALF (<0.25 mg/L) and very low drug levels in lung tissue (0.28 ± 0.02 and 0.40 ± 0.11 μg/g for 60 rspm; 2 mL of tidal volume and 30 rspm; 4 mL of tidal volume, respectively).

Finally, Table 2 shows the BALF/EF, lung/EF and BALF/ lung mean partition coefficients of linezolid for the two administration modes and both respiratory patterns. Since EF after inhalatory route and BAL after systemic administration were under quantification limit partition coefficients for these conditions were not calculated.

Discussion

The results of the present study reveal that inhalation delivery of linezolid might be an excellent strategy to increase drug levels in pulmonary fluid and tissue without significant increases in systemic exposure to the antibiotic. Also, the influence of the respiratory mode on pulmonary disposition is demonstrated and

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Table 1. Mean pharmacokinetic parameters of linezolid in lung after systemic administration

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>60 rspm 2 mL tv (mean ± SD)</th>
<th>30 rspm 4 mL tv (mean ± SD)</th>
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</thead>
<tbody>
<tr>
<td>AUC0–1 (mg h/L)</td>
<td>1.48 ± 0.20</td>
<td>1.49 ± 0.25</td>
</tr>
<tr>
<td>MTT (min)</td>
<td>0.47 ± 0.06</td>
<td>0.62 ± 0.09*</td>
</tr>
<tr>
<td>V/Lw (mL/g)</td>
<td>1.81 ± 0.34</td>
<td>2.97 ± 0.26*</td>
</tr>
<tr>
<td>Kw (s⁻¹)</td>
<td>0.018 ± 0.003</td>
<td>0.012 ± 0.006</td>
</tr>
</tbody>
</table>

AUC, area under the concentration–time curve; MTT, mean transit time; V, distribution volume; Lw, lung weight; Kw, washout rate constant; tv, tidal volume; rspm, respirations per minute.

*P < 0.05.
Table 2. BALF/EF, lung/EF and BALF/lung mean partition coefficients of linezolid

<table>
<thead>
<tr>
<th>Partition coefficients</th>
<th>Systemic delivery</th>
<th>Pulmonary delivery</th>
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<tbody>
<tr>
<td></td>
<td>60 rspm 2 mL tv</td>
<td>30 rspm 4 mL tv</td>
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<tr>
<td></td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
</tr>
<tr>
<td>BALF/EF</td>
<td>—</td>
<td>&gt;24.17</td>
</tr>
<tr>
<td>Lung tissue/EF</td>
<td>0.92 ± 0.257</td>
<td>24.17 ± 9.98</td>
</tr>
<tr>
<td>BALF/lung tissue</td>
<td>—</td>
<td>&gt;8.33</td>
</tr>
</tbody>
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EF, efferent fluid; BALF, bronchoalveolar lavage fluid; tv, tidal volume; rspm, respirations per minute.

*P < 0.05.

an improvement of drug transfer through the alveolar–epithelium barrier for deeper and slower respiration can be confirmed, since the BALF/EF and lung tissue/EF concentration ratios were higher for the 4 mL of tidal volume and 30 rspm than those obtained for 2 mL and 60 rspm (Table 2). Pulmonary delivery of linezolid to isolated lung leads to very high levels of the antibiotic in BALF and lung tissue, whereas systemic administration elicits moderate levels in those pulmonary samples. The BALF/EF concentration ratio was 24.17 ± 9.98 for pulmonary route and the lung tissue/EF ratio was also much higher for inhalation (8.33 ± 2.51) than with systemic administration (1.90 ± 0.78), all these values corresponding to the 4 mL of tidal volume and 30 rspm and the differences revealing statistical significance. Our results from systemic administration are in concordance with most of the reported data concerning the pulmonary distribution of linezolid after standard treatment, providing ELF/serum concentration ratios of 0.8–1.8,9–11 although they differ significantly from the results of Conte et al.,7 who found BALF/plasma concentration ratios as high as 4.1–2.4, or the data from Gentry-Nielsen et al.,1 who found BALF/plasma concentration ratios as low as 0.2 in rats. Differences in the methodological procedures used for sampling and the estimation of concentrations are probably responsible for the large differences encountered among the data reported. However, except for the two latter mentioned studies showing these extreme values, the data on linezolid pulmonary distribution reveal a moderate capacity of the drug to reach and become accumulated in lung spaces with the standard dosage regimens.

Interest in pulmonary drug delivery is increasing progressively with the appearance of the new generation of drugs obtained with biotechnological procedures owing to the instability in the gastrointestinal environment of this type of drug. An extensive absorption area, a reduced thickness of the alveolar epithelium, high vascularization and limited metabolic capacity all contribute to a rapid systemic access and pharmacological response together with a dosage reduction and the avoidance of invasive routes leading to patient discomfort. Pulmonary administration also provides an efficient method for the passive targeting of drugs used in pulmonary diseases. This route is currently a reality for asthma treatment but not for respiratory infections. The nebulization of antibiotics seems to have relevant advantages over other administration modes: drug access to all regions of the respiratory system and the avoidance of extensive systemic drug exposure (leading to a lower incidence of side effects) are some of the most interesting features. The results of the present study reinforce the foregoing aspects. Since an increase of serum drug levels or an extension of the treatment period are both related to a higher incidence of side effects for linezolid,12,15 administration by inhalation might provide an alternative approach to achieve higher levels of the antibiotic in the pulmonary space without increasing systemic exposure and hence with no risk of increasing the incidence of side effects.

The paucity of information about relevant topics such as the optimum dose, the pharmacokinetics, the potential increase in patient sensitivity or the influence of respiratory patterns on drug disposition are probably the main reason for the current restricted application of this administration route for pulmonary infections. Thus, experimental and clinical assays aimed at providing this type of information would be of great interest and would be helpful for deciding whether the inhalation route proposed for linezolid might contribute to the improvement of clinical outcomes.

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Transparency declarations

None to declare.

References


