

Pharmacokinetics and Pulmonary Disposition of Tedizolid and Linezolid in Three Murine Models

Rebecca A. Keef¹, Jared L. Crandon¹, David P. Nicolau^{1,2}
¹Center for Anti-Infective Research & Development, Hartford Hospital, ²Division of Infectious Diseases, Hartford Hospital, Hartford, CT, USA

David P. Nicolau, Pharm.D., F.C.C.P., F.I.D.S.A.
 Center for Anti-Infective R&D
 Hartford Hospital
 80 Seymour Street
 Hartford, CT 06102
 E-mail: dnicola@hartosp.org

ABSTRACT

Background: When evaluating the *in vivo* pharmacodynamics of antimicrobials, comparative efficacy in neutropenic (-) and immunocompetent (+) animals is often used to evaluate the effect of the host immune system. Assumptions made between pharmacokinetic (PK) exposures and drug disposition across models may lead to inaccurate comparisons. We evaluated and compared the effect of infection and immune status on the PK of tedizolid (TR-700) and linezolid (LZD).

Methods: Tedizolid phosphate (formerly known as torezolid phosphate or TR-701) 8.4 mg/kg and LZD 60 mg/kg were administered as single doses to -, +, and uninfected BALB/c mice. Blood and bronchoalveolar lavage (BAL) samples were collected from 6 mice at 1, 2, 4, 8, 12, and 24 h for TR-700 and at 1, 2, 4, 8, and 12 h for LZD. Drug concentrations in plasma and BAL fluid were determined by LC-MS/MS and HPLC for TR-700 and LZD, respectively; the urea correction method was used to determine epithelial lining fluid (ELF) concentrations. Drug penetration was calculated as the ratio of the 24 h area under the concentration-time curve (AUC₀₋₂₄) in ELF to the free AUC₀₋₂₄ (AUC₀₋₂₄) in plasma.

Results: PK exposures and relative penetration ratios are shown in the table. For both TR-700 and LZD, + mice had the highest plasma exposures whereas the uninfected mice had the lowest. Additionally, irrespective of immune status, TR-700 had enhanced ELF penetration in comparison with LZD.

Drug	Model	Plasma AUC ₀₋₂₄	ELF AUC ₀₋₂₄	ELF Penetration Ratio
Tedizolid 8.4 mg/kg	Immunocompetent Infected	4.71	43.85	9.32
	Neutropenic Infected	3.35	35.64	10.64
	Immunocompetent Uninfected	2.77	16.99	6.13
Linezolid 60 mg/kg	Immunocompetent Infected	130.49	167.29	1.28
	Neutropenic Infected	52.71	46.08	0.87
	Immunocompetent Uninfected	36.22	62.48	1.72

Conclusions: The presence of lung infection and immune status resulted in differences in plasma and pulmonary profile of TR-700 and LZD. While the magnitude of these differences varied between agents, the trend was consistent. TR-700 exhibited improved penetration into the ELF in comparison with LZD regardless of immune competency or infection status. Moreover, the presence of infection improved penetration for TR-700. These data emphasize the importance of PK confirmation in each model.

INTRODUCTION

- Tedizolid, the active moiety of tedizolid phosphate, is a novel oxazolidinone with activity against Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus*.
- Linezolid is the only other FDA approved oxazolidinone thus there has been much interest in comparing the *in vitro* and *in vivo* efficacy of these two oxazolidinones.
- When assessing the *in vivo* pharmacodynamics of antimicrobials, neutropenic and immunocompetent infection models are often used to evaluate the degree of antibacterial activity of a given regimen as well as the impact of the host immune system on bacterial clearance.
- Frequently, drug exposures are evaluated in only one model and it is assumed that the pharmacokinetic profile is similar in each of these models.
- Without pharmacokinetic confirmation, exposure disparities rather than the host immune system may actually be responsible for any differences in efficacy leading to inaccurate comparisons between models and/or the compounds under investigation.

OBJECTIVE

- To evaluate and compare the effect of lung infection and immune status on the pharmacokinetics and pulmonary disposition of tedizolid and linezolid.

METHODS

Antimicrobial Agents

- Analytical grade tedizolid phosphate (Albany Molecular Research Inc., Albany, NY) and linezolid (Pfizer Inc., Groton, CT) were used for the *in vivo* analyses.
- Each antimicrobial was weighed, reconstituted, and further diluted in appropriate diluents to achieve the desired concentration.
- Solutions were stored under refrigeration and discarded 24 hours after reconstitution.

Murine Models

- Specific-pathogen free, female Balb/c mice weighing approximately 20 g each were utilized throughout these experiments.
- Neutropenic Infected:** Mice were rendered transiently neutropenic by intraperitoneal injections of cyclo-phosphamide (Baxter, Deerfield, IL) 250 mg/kg and 100 mg/kg given four and one day(s), respectively, prior to inoculation.
 - Six hours prior to the initiation of antimicrobial therapy, isoflurane anesthetized mice were held upright and orally inoculated with 0.05 mL of a 10⁷ CFU/mL suspension of the *S. aureus* 156 in 3% mucin (Sigma-Aldrich, St. Louis, MO).
 - Inocula were administered directly into the buccal cavity of the mice and their nares were blocked to induce aspiration.

- Immunocompetent Infected:** Mice underwent the same procedure as neutropenic mice, but without the use of cyclophosphamide prior to inoculation with an inoculum of 10⁹ CFU/mL.
- Immunocompetent Uninfected:** No procedures were performed prior to dose administration.

Pharmacokinetic studies

- Single doses of tedizolid 8.4 mg/kg or linezolid 60 mg/kg were administered.
- Blood and bronchoalveolar lavage (BAL) fluid was collected from groups of 6 mice at 5 – 6 timepoints over the 12 – 24 hour dosing interval in each of the murine models.
- Plasma (tedizolid) or serum (linezolid) samples, hereafter referred to as blood, were separated by centrifugation and stored at -80°C until analysis.
- Concentrations were analyzed by a validated liquid chromatography-tandem mass spectrometry (LC-MS-MS) and high performance liquid chromatography (HPLC) assay for tedizolid and linezolid, respectively.
- Protein binding values utilized for tedizolid and linezolid were 85% and 30%, respectively^{1,2,3}.
- Area under the free drug concentration-time curve (AUC) for the both regimens was calculated using the trapezoidal rule.
- Portions of blood and BAL fluid were tested for the urea concentration by a commercially available urea assay (Teco Diagnostics, Anaheim CA).
- Drug concentrations in epithelial lining fluid (ELF) were calculated from the following formula: ELF concentration = BAL concentration x (blood urea concentration / BAL urea concentration).

RESULTS

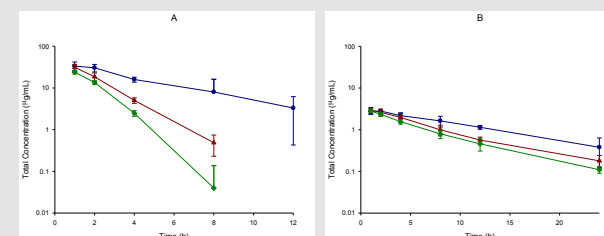
- Pharmacokinetic exposures and relative penetration ratios are presented in Table 1.
- Concentration-time profiles for each agent for all model conditions are shown in Figure 1.
- Blood pharmacokinetic exposures for both tedizolid and linezolid, respectively, were consistently the highest in the immunocompetent model and the lowest in the uninfected model.
 - For tedizolid, exposures in the immunocompetent and uninfected models were 41% higher and 17% lower than what was observed in the neutropenic model.
 - As for linezolid, more pronounced trends were noted with exposures increased by 150% in the immunocompetent model and decreased by 31% in the uninfected model.
- ELF exposures for both drugs were highest in the immunocompetent animals.
- Tedizolid had enhanced ELF penetration for all three models in comparison with linezolid.
- The presence of infection improved penetration for tedizolid (penetration ratio, 9.32 – 10.62 for infected vs. 6.13 for uninfected); whereas, linezolid had enhanced penetration in the uninfected animals (penetration ratio, 0.87 – 1.28 for infected vs. 1.72 for uninfected).

Table 1. Pharmacokinetic and relative penetration ratios for single doses of tedizolid 8.4 mg/kg and linezolid 60 mg/kg in three murine models.

Drug	Model	Plasma AUC ₀₋₂₄	ELF AUC ₀₋₂₄	ELF Penetration Ratio
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AUC₀₋₂₄, area under the free drug concentration-time curve over 24 hours from a single dose; ELF, epithelial lining fluid

Figure 1. A) Serum concentration-time profile for a single dose of linezolid 60 mg/kg in the immunocompetent infected (blue circles), neutropenic infected (red triangles), and immunocompetent uninfected (green diamonds) murine model. B) Plasma concentration-time profile for a single dose of tedizolid 8.4 mg/kg in the immunocompetent infected (red triangles), neutropenic infected (blue circles), and immunocompetent uninfected (green diamonds) murine model.



CONCLUSIONS

- Immune status and the presence of lung infection resulted in discordances in the blood and pulmonary profiles of tedizolid and linezolid.
 - While the dosing regimens of tedizolid 8.4 mg/kg every 24 hours and linezolid 60 mg/kg every 12 hours may achieve similar exposures as humans in the neutropenic Balb/c mouse, substantially higher exposures were attained in the immunocompetent infected model and lower exposures in uninfected mice.
 - The impact of immune status and infection appears to have a more profound effect on the pharmacokinetic profile of linezolid versus that observed with tedizolid.
- Assumptions made regarding similar pharmacokinetic exposures and drug disposition between models would lead to inaccurate efficacy comparisons if these dosing regimens were employed in these models.
- These substantial differences in exposures due to murine model emphasize the importance of pharmacokinetic confirmation in each murine model utilized.

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