Comparison of tigecycline penetration into the epithelial lining fluid of infected and uninfected murine lungs

Jared L. Crandon¹, Aryun Kim¹ and David P. Nicolau¹,²*

¹Center for Anti-Infective Research and Development, Hartford Hospital, Hartford, CT, USA; ²Division of Infectious Diseases, Hartford Hospital, Hartford, CT, USA

Received 9 June 2009; returned 22 July 2009; revised 24 July 2009; accepted 27 July 2009

Objectives: When evaluating the pharmacodynamics of antimicrobials, assumptions are often made relative to their pharmacokinetics. One example of this is applying tissue penetration results of uninfected hosts to those displaying a targeted illness. As tigecycline evolves into a potential treatment option for pneumonia, we determined whether the presence of a lung infection affected the penetration of the drug into the epithelial lining fluid (ELF).

Methods: Single doses of tigecycline 50 and 25 mg/kg were administered to neutropenic ICR mice with or without the presence of an Acinetobacter baumannii lung infection. Serum samples were gathered at 0.5–24 h after tigecycline administration; bronchoalveolar lavage was conducted at 1, 1.5, 4 and 8 h. Tigecycline concentrations were determined by HPLC. Comparisons of ELF penetration in infected and uninfected lungs were based on the ratios of the AUC₀–₈ in ELF and the free AUC₀–₈ in serum. AUCs were calculated by the trapezoidal rule.

Results: The group without pulmonary infection displayed an ELF penetration ratio of 8.1 and 6.2 for the 50 and 25 mg/kg doses, respectively. The respective penetration ratios in the infected lungs were 23.3 and 12.9.

Conclusions: While tigecycline exhibits excellent ELF penetration in healthy and infected murine lungs, the presence of infection greatly enhances penetration. Moreover, increased systemic exposures of tigecycline result in greater ELF penetration, regardless of infection status. When future tigecycline clinical trials for the treatment of pneumonia are considered, escalated doses may reap greater than expected benefits towards achieving adequate pharmacodynamic indexes within the lungs.

Keywords: bronchopulmonary, pneumonia, pharmacokinetics

Introduction

While significant advances in the concepts of antimicrobial pharmacokinetic and pharmacodynamic theory have been made over the last few decades, there are still many questions that remain. One important question left relatively unanswered is: how does the presence of an infection, or, better, the location of an infection alter site-specific pharmacokinetics? No data attempting to answer this question are available for the glycylcycline antibiotic tigecycline and data available for other antimicrobials are sparse. Herein, we administered tigecycline to neutropenic mice with and without pulmonary infections to assess the impact of lung infection on bronchopulmonary pharmacokinetics.

Materials and methods

The study was reviewed and approved by the Hartford Hospital Institutional Animal Care and Use Committee. ICR mice (Harlan Sprague Dawley, Inc., Indianapolis, IN, USA) were rendered transiently neutropenic with intraperitoneal doses of cyclophosphamide (Baxter Healthcare Corporation, Deerfield, IL, USA) 1 and 4 days prior to bacterial inoculation.¹ Lung infection was established using a 10⁷ cfu/mL inoculum of a clinical Acinetobacter baumannii isolate made from fresh subcultures in normal saline and 3% mucin (Sigma-Aldrich, St Louis, MO, USA). Isoflurane-anaesthetized mice were orally administered 0.05 mL of this solution and their nostrils were blocked until the fluid was aspirated. Mice not infected in the pulmonary compartment were inoculated

*Corresponding author. Center for Anti-Infective Research and Development, Hartford Hospital, 80 Seymour Street, Hartford, CT 06102, USA. Tel: +1-860-545-3941; Fax: +1-860-545-3992; E-mail: dnicola@harthosp.org

© The Author 2009. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oxfordjournals.org
in the thigh and served as comparators. At 8–12 timepoints ranging from 0.5 to 24 h after tigecycline (laboratory grade tigecycline; Wyeth, Madison, NJ, USA) administration, groups of six mice were euthanized via CO2 inhalation and blood was collected via cardiac puncture. In addition, at the 1, 1.5, 4 and 8 h timepoints a bronchoalveolar lavage (BAL) was performed after blood collection and the lavage sample was centrifuged to obtain epithelial lining fluid (ELF) using methods previously described. In all studies, tigecycline was administered as single subcutaneous injections of 25 or 50 mg/kg. Tigecycline concentrations were evaluated in serum and lavage fluid using a validated HPLC assay. Interday and intraday coefficients of variation were <5%. Tigecycline concentrations in the ELF (TIG ELF) were calculated using the formula: TIGELF = TIGlavage x (ureaserum/urealavage), where ureaserum and urealavage are the concentrations of urea in the serum and lavage fluid, respectively, and TIGlavage is the concentration of tigecycline in the lavage fluid. The respective AUC0–8 were calculated using the trapezoidal rule. While tigecycline exposures in the lung were assumed to consist of unbound drug only, free serum exposures (f) were calculated using the concentration-dependent protein binding previously described. The degree of penetration into the ELF was determined by comparing the AUC0–8 of ELF with the fAUC0–8 of serum. Statistical comparisons between penetration ratios were determined by a Student’s t-test or Mann–Whitney U-test if data were not normally distributed. A P value of <0.05 was considered significant.

Results

Serum pharmacokinetics were best described using a two-compartment model with first order input and elimination. Tigecycline concentrations in ELF and serum for mice with and without pulmonary infection at each of the BAL timepoints are shown in Figure 1. Table 1 displays the ELF penetration ratios for each dose in mice with and without pulmonary infection. Penetration was statistically greater in mice with infected lungs relative to those without a lung infection (P<0.001). Within each group of mice, penetration was statistically greater for the 50 mg/kg dose when compared with the 25 mg/kg dose (P<0.025).

Discussion

Herein, we described the ability of tigecycline to penetrate the lungs of mice with or without the presence of a lung infection and showed vast increases in the group with infected lungs. No similar studies using tigecycline exist and the limited data available for other classes of antimicrobials vary. A previous assessment comparing fluoroquinolone penetration into ELF of healthy mice and those whose lungs were infected showed no difference between groups, nor did a similar study utilizing ceftriaxone. In contrast, however, endotoxin exposure-mediated airway inflammation in rats produced an increase in ELF penetration of aminoglycosides when compared with unexposed animals.

Pharmacokinetic data determined from healthy volunteers receiving 50 mg of tigecycline every 12 h demonstrated an ELF penetration ratio of 1.3. If the serum AUC is adjusted to account for protein binding (79%), as was done in the current analysis, penetration increases to 6.28, a value similar to those seen in our animals without pulmonary infections. Given the results of our first in vivo assessment of tigecycline ELF penetration in a lung infection model, it seems reasonable to suggest that penetration into the lungs of patients with lower respiratory...
Intrapulmonary pharmacokinetics of tigecycline

tract infections may be greater than that reported in healthy volunteers. While these findings are seemingly conflicting with the results of a recent Phase 3 trial evaluating tigecycline and imipenem for the treatment of hospital-acquired pneumonia, it should be noted that the subset of patients that faired poorly in that analysis were those suffering from ventilator-associated pneumonia, a subset of patients often infected with the most resistant populations of bacteria. Accordingly, although lung exposures in infected patients may be greater than those reported in healthy volunteers, they may yet fall short of the targets required for maximal efficacy, especially for organisms on the upper end of the MIC distribution.

Another interesting observation made in this analysis was that ELF penetration increased with increasing tigecycline dose. While ample data exist describing the ELF penetration of various antimicrobials, very few evaluate differing dosages. Penetration data for the novel ketolide antibiotic cethromycin in healthy volunteers displayed the inverse of our results, with ELF penetration ratios decreasing from 12.6 to 7.9 when daily doses were increased from 150 to 300 mg, respectively. Similarly, an analysis of meropenem intrapulmonary pharmacokinetics in healthy volunteers revealed a decrease in the ELF penetration ratio of 0.43 to 0.28 with doses of 0.5 and 1 g, respectively. While at present the reasons behind these observations and their impact on clinical practice are not well explained, they pave the way for future studies to help elucidate the mechanism and influence of such phenomena.

When calculating the penetration ratios of tigecycline into ELF in this analysis, we assumed that the tigecycline recovered in the ELF was free drug and thus compared this with free drug concentrations within the serum. This assumption was made for several reasons. First, it is unclear what amounts of protein are actually available within the pulmonary compartment for tigecycline binding. Secondly, even if proteins are available in the ELF, the affinity of tigecycline to bind within this compartment is also undescribed. Lastly, while the magnitude of the penetration ratio values decreased when calculated using total drug AUCs in both serum and ELF, the statistical conclusions were similar to those noted for free drug calculations (data not shown).

In this analysis, we evaluated the penetration of tigecycline into ELF with and without the presence of a lung infection. We concluded that while tigecycline penetrates well into the ELF of both healthy and infected murine lungs, penetration is significantly better in infected lungs. Moreover, an increase in systemic exposure provided an enhancement in ELF penetration and occurred with or without the presence of a lung infection. These data support the notion that tigecycline exposures in the lungs of infected patients are probably higher than those predicted in healthy volunteers. Additionally, there is the potential that escalated doses may reap greater than expected benefits towards achieving adequate exposures within the lungs. Lastly, caution should be exercised when relying on the modelling of intrapulmonary data based solely on the pharmacokinetics of healthy volunteers and/or a single dosing regimen to guide pharmacodynamic-based novel dose selection for patients.

Funding

This study was supported by a research grant from Wyeth Research, Madison, NJ, USA.

Transparency declarations

None to declare.

References