Evaluation of Tigecycline Penetration into Colon Wall Tissue and Epithelial Lining Fluid Using a Population Pharmacokinetic Model and Monte Carlo Simulation

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The objective of these analyses was to assess the penetration of tigecycline into colon wall tissue and epithelial lining fluid (ELF). The analyses included data from subjects without infection (phase 1) and patients with intra-abdominal infections (phase 2/3). Steady-state serum samples were collected from all subjects/patients (n = 577), while colon wall specimens (n = 23) and ELF specimens (n = 30) were obtained from subjects without infection. Tissue and serum data were simultaneously comodeled by using the BigNPAG program, and a four-compartment, open model with zero-order intravenous input and first-order elimination was employed. To examine the full range of tissue penetration and the associated probabilities of occurrence, a 9,999-subject Monte Carlo simulation was performed with two outputs, one for ELF penetration and one for colon wall tissue penetration. Data were well fit using models described above, with all \( r^2 \) values above 0.95. For subjects without infection, the median (5th and 95th percentiles) colon wall and ELF penetration ratios were 1.73 (0.160 and 199) and 1.15 (0.561 and 5.23), respectively. Simulation results predict that tissue penetration varies considerably and likely explain unexpected clinical outcomes for those patients infected with strains at margins of the MIC distribution.

For an antibacterial agent to be clinically effective, it must reach the infection loci in concentrations sufficient to bind a critical fraction of bacterial receptor sites and remain there long enough to inhibit a cellular process that is essential for bacterial life. Thus, determining the level of drug exposure in tissue compartments is helpful for gaining a better understanding of the relationship between drug exposure and response in circumstances where tissue infections are being treated. However, determining drug exposure for human tissue is fraught with difficulties.

The main difficulties relate to study design and data analysis methods. In most circumstances, it is not technically feasible to obtain enough tissue samples from a single patient to fully characterize drug exposure. In fact, most tissue penetration studies collect only a single tissue sample per patient (with a matched plasma sample), often during a major surgical intervention. Traditional analyses of such data involve the calculation of tissue-plasma ratios, the validity of which is suspect given the time-dependent nature of both the tissue and plasma concentrations and the observation that antimicrobial agents display multicompartmental pharmacokinetic profiles, which are evidence of different rates of distribution into and out of different tissues. For most drugs, the concentration-time profiles in plasma and tissue compartments differ in both shape and magnitude. This difference leads to system hysteresis, and the ratio of drug in the tissue to that in the plasma changes with time. Therefore, the clinical relevance of calculated tissue-plasma ratios is often difficult to ascertain.

One way to overcome this limitation is through population pharmacokinetic analysis, which can be utilized to model simultaneously all the data (plasma and tissue concentrations) from all the subjects in a study. The mean parameter values and their dispersions, in conjunction with Monte Carlo simulation, can be used to estimate the range and likelihood of exposure at the effect site. Such an approach has been used previously to characterize drug penetration into prostate and epithelial lining fluid (ELF) (5, 6).

In this work, we used population pharmacokinetic analysis to evaluate tigecycline penetration into ELF and colon wall tissue. Tigecycline is the first in a new antimicrobial class, the glycyclines, to receive approval from the Food and Drug Administration. Tigecycline is generally well tolerated and has proven effective for the treatment of complicated skin, skin structure, and intra-abdominal infections (Tygacil for injection package insert; Wyeth Pharmaceuticals, Inc., Philadelphia, PA).

MATERIALS AND METHODS

Patients, clinical study design, and drug assay. Demographic and pharmacokinetic data were obtained from two phase 1 studies (studies 1 and 2), one phase 2 study (study 3), and two phase 3 studies (studies 4 and 5). Study 1 included healthy volunteers (nonsmoking men and women) aged 18 to 55 years, with body mass index values (BMI) of 18 to 30 kg/m²; these subjects could not have significant comorbidities nor could they be receiving drugs or supplements of any kind (with the exception of occasional acetaminophen or vitamins). Study 2 included subjects aged more than 18 years who were scheduled for lung, colon, gallbladder, or bone surgery or who had a scheduled or unscheduled lumbar
puncture; subjects were excluded if they had conditions that hindered the obtaining of appropriate tissue samples. The phase 2 and 3 studies (studies 3 to 5) included hospitalized patients with complicated intra-abdominal infections; study 3 was an open-label, multicenter study, and studies 4 and 5 were double-blind, comparative studies. The full details of these studies have been presented elsewhere previously (11, 16).

In study 1, subjects received a 100-mg loading dose of intravenous (i.v.) tigecycline on day 1, followed by a 50-mg maintenance dose of i.v. tigecycline every 12 h thereafter through day 4, for a total of seven doses. All doses were administered by i.v. infusion for over 30 min. Venous blood samples for tigecycline concentration analysis were collected before the first dose on day 1 (the sample was not used for the analysis), before the last dose on day 4, and at hours 0.5 (end of infusion), 1, 2, 3, 4, 6, 8, 12, and 24 after the start of the infusion of the last dose on day 4. A bronchoalveolar lavage (BAL) was scheduled once for each subject at a specified time after the last (seventh) dose of tigecycline on day 4. Six time points for BAL were designated, with a total of five subjects for each subject at a specified time after the last (seventh) dose of tigecycline on day 4. A separate bronchoalveolar lavage fluid (BALF) was collected immediately before the BAL. A separate urea nitrogen sample was drawn from the lavage fluid during BAL. Concentrations of tigecycline in serum and BAL fluid were determined by sensitive and specific liquid chromatography-time of flight mass spectrometry methods (3, 9). The concentration of tigecycline in the lung epithelial lining fluid (C\text{ELF}) was calculated as \( C\text{BAL} \cdot (V\text{BAL}/V\text{ELF}) \), where \( C\text{BAL} \) is the concentration in the BAL fluid, \( V\text{BAL} \) is the volume of the aspirated BAL fluid, and \( V\text{ELF} \) is the volume of lung ELF. The volume of the lung ELF within the BAL fluid was estimated by the formula \( V\text{ELF} \cdot (\text{urea BAL/urea serum}) \), where urea BAL and urea serum represent the concentrations of urea in the BAL fluid and serum, respectively (2).

For study 2, all subjects received a single 100-mg dose of i.v. tigecycline administered over approximately 30 min. The tigecycline infusion was to be started approximately 4, 8, 12, or 24 h before collection of the tissue samples according to the following schedule: six subjects from each tissue/fluid group were to be assigned to each of the four time points from the start of the infusion of tigecycline (approximately 4, 8, 12, or 24 h before the collection of the tissue samples). Twenty-four subjects were enrolled in each of the tissue/fluid-type groups (lung, colon, gallbladder, bone, and cerebral spinal fluid). According to the tissue/fluid types to be collected during the procedures for which the subjects were scheduled, venous blood samples for tigecycline concentration analysis were to be collected before the infusion of tigecycline (time zero), at the end of the infusion (approximately 30 min after the start of the infusion), and at the time corresponding to the tissue/fluid sample collection. Concentrations of tigecycline in serum and tissues/fluids were determined by sensitive and specific liquid chromatography-time of flight mass spectrometry methods (12). Sample processing was as follows (12): (i) a 0.20-g aliquot of tissue was mixed with 3 ml of internal standard solution ([\( \text{\text{-}butyl d9} \)] tigecycline, 0.03 mg/liter in acetonitrile); (ii) samples were homogenized using a tissue homogenizer (Tissue-Tearor model 398; BioSpec Products, Inc., Bartlesville, OK); and (iii) after centrifugation, the supernatant was transferred to a clean culture tube and evaporated to dryness at room temperature under vacuum.

The low-, middle-, and high-quality control concentrations for some tissue samples exhibited acceptable precision and accuracy levels (5% and 108%, respectively, at 0.022 mg/kg; 5% and 98%, respectively, at 0.5 mg/kg; and 2% and 102%, respectively, at 1.5 mg/kg) (12).

In studies 3, 4, and 5, patients received 100-mg loading doses of i.v. tigecycline on day 1, followed by 50-mg maintenance doses of i.v. tigecycline every 12 h for at least 5 days but not more than 14 days. Venous blood samples for tigecycline concentration analysis were collected before or on the day of discharge. Samples were drawn at the following times: just prior to the dose, at the end of the infusion (30 min or 1 h, depending on the study), and 3 and 6 h after the start of the infusion.

Pharmacokinetic analyses and Monte Carlo simulation. A single model was constructed to describe the serum, ELF, and colon wall pharmacokinetics. The ELF concentrations from study 1 and colon wall concentrations from study 2 were comodeled with the serum samples from all five studies. Tissue and serum data were simultaneously comodeled by using the BigNPAG program (14); a four-compartment, open model with zero-order i.v. input and first-order elimination was employed. A specific volume term was used for the estimation of volumes in all of the tissue compartments. Bayesian estimates were obtained by using the population-of-one utility within BigNPAG.

Estimates of drug exposure in serum and tissues were obtained using two methods: direct simulation using only the mean parameter vector and Monte Carlo simulation using the mean parameter vector and the distribution of parameter estimates. For the latter method, a 9,999-subject Monte Carlo simulation was performed; the mean parameter vector from the BigNPAG analysis was embedded in subroutine PRIOR of the ADAPT II package of programs of D’Argenio and Schumitzky (University of Southern California) (4). The distribution of parameter estimates was assumed to be log normal. Penetration ratios were calculated as the ratio of the area under the concentration-time curve over 24 h at steady state (AUCs24h) in tissue to the AUCs24h in serum.

### RESULTS

Demographics. As shown in Table 1, subjects from whom ELIF was collected (study 1) were younger and more likely to be male than were uninfected subjects from whom colon wall tissue was collected (study 2) and patients with intra-abdominal infections (studies 3 to 5). Mean patient weight values were similar regardless of study cohort.

**Pharmacokinetic parameter values.** Mean parameter values and standard deviations from the pharmacokinetic models are shown in Table 2. Serum clearance is modestly lower than that observed in previous analyses (15); this difference may reflect differences in demographics and/or disease state between studies. Clearly there was a high degree of interindividual variability in tissue pharmacokinetics, as evidenced by the high standard deviations.

Figure 1 shows the regression of observed-versus-predicted tigecycline serum (\( n = 1,655 \)), colon wall tissue (\( n = 23 \)) and ELIF (\( n = 13 \)) concentrations for the final model. Of the 30 ELIF samples obtained, 17 had concentrations below the limit of quantification; the mean time since the last dose for the below-the-limit-of-quantitation samples in ELIF was 12 h. For serum and for colon wall tissue, regression line intercepts for observed-versus-predicted concentration values were \(-0.031\) and \(+0.041\), respectively, and their \(r^2\) values were both greater than 0.9.
than 0.95 ($P < 0.001$). For ELF, the fit was less robust overall (regression line intercept = 0.096; adjusted $r^2 = 0.55; P = 0.002$). However, if the one outlier is removed (observed = 0.29; predicted = 0.88), the fit is in agreement with that seen in the other matrices (regression line intercept = 0.014; adjusted $r^2 = 0.95; P < 0.001$).

**Tissue penetration and Monte Carlo simulation.** Table 3 shows tigecycline exposure in serum and tissue as well as penetration ratios for colon wall tissue and ELF. For subjects without intra-abdominal infection, the median ratio for colon wall tissue penetration was 1.73 and the interquartile range was 0.683 to 3.91. The median ratio for ELF penetration was 1.15, while the interquartile range of this penetration ratio was 0.823 to 2.45. Figure 2 shows the frequency distribution histogram of penetration ratios as predicted from the 9,999-patient Monte Carlo simulation. The flat nature of this distribution illustrates the extreme variability in ELF/tissue penetration such that in the absence of prior information regarding ELF/tissue concentrations in a given patient, the patient would be as likely to have a penetration ratio of 0.1 as he or she would to have a penetration ratio of 6.0.

**DISCUSSION**

There are three major findings of these analyses, each of which is instructive with regard to the clinical pharmacology of tigecycline. First, median tigecycline exposure in colon wall tissue of subjects without infection is significantly higher (173%) than that in serum. Second, tigecycline exposure in the ELF of subjects without infection is similar (117%) to that in serum samples. Third, there is considerable variability predicted in effect site exposure, as evidenced by the dispersion around the median estimates of the penetration ratio (Fig. 2). Each of these insights comes with important caveats and implications.

Although tigecycline penetration into colon wall tissue of subjects without infection is high, it is important to keep in mind that the median tissue penetration ratio described herein was based on a small sample of colon wall determinations from subjects without infection ($n = 23$). However, given the pathophysiology of intra-abdominal infection, tigecycline exposure in colon wall tissue is likely still higher in the target patient population. Patients with intra-abdominal infection classically have considerable inflammation, often due to the disease state and surgical trauma. Also, the concentration of tigecycline in white cells may play a role in drug delivery to the infection site in the infected patient.

Tigecycline exposure in ELF was approximately 117% of that in serum. Given that tigecycline is 71% to 89% bound to serum proteins (Tygacil for injection package insert; Wyeth Pharmaceuticals, Inc., Philadelphia, PA), the exposure in ELF is greater than the free-drug exposure in serum. This observation is not novel (6, 13). For instance, in an analysis that utilized mathematical techniques similar to those described herein, the median ELF-plasma penetration ratio of levofloxacin was 1.43 (6).

It is also critical to acknowledge that the subjects enrolled in the ELF study were not suffering from acute lung infection, and thus, the effect of pulmonary inflammation on the penetration of tigecycline into ELF could not be evaluated. Acute lung infection can alter the penetration of a drug into ELF. There are several reasons for this observation, including drug trafficking via macrophages (10) and changes in the permeability of alveolar capillary membrane protein (8). The former...
explanation classically applies to antimicrobial agents that concentrate within macrophages, like macrolides and, to a lesser extent, quinolones and tetracyclines, while the latter applies to essentially all agents. The effect of lower lung infection on tigecycline penetration has yet to be examined, but it is highly likely that tigecycline exposure in the ELF of patients with pneumonia is greater than that reported herein. There was considerable variability in effect site exposure, as

TABLE 3. Tigecycline exposure in serum and penetration into colon wall tissue or ELF for subjects with tissue concentration samples

<table>
<thead>
<tr>
<th>Percentile</th>
<th>Colon wall tissue</th>
<th>ELF</th>
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<tbody>
<tr>
<td></td>
<td>( \text{AUC}_{0-24, \text{serum}} )</td>
<td>( \text{AUC}_{0-24, \text{colon wall}} )</td>
</tr>
<tr>
<td>5th</td>
<td>4.89</td>
<td>1.65</td>
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<tr>
<td>25th</td>
<td>6.09</td>
<td>6.44</td>
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<tr>
<td>50th</td>
<td>6.57</td>
<td>15.6</td>
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<tr>
<td>75th</td>
<td>9.93</td>
<td>30.4</td>
</tr>
<tr>
<td>95th</td>
<td>112</td>
<td>3,367</td>
</tr>
</tbody>
</table>

\(^a\) For colon wall tissue and ELF, AUC results are shown as mg · h/liter. For colon wall tissue, \(n\) was 23; for ELF, \(n\) was 13.

FIG. 2. Frequency distribution histogram of predicted penetration ratios for tigecycline in colon wall tissue (A) and ELF (B) derived from the Monte Carlo simulation.
evidenced by the greater dispersion around the point estimates of exposure (AUC\(_{0-24}\)) in colon wall tissue (median [25th to 75th percentile] = 15.6 [6.44 to 30.4] mg · h/liter) than for that in serum (median [25th to 75th percentile] = 6.57 [6.09 to 9.93] mg · h/liter). There are two reasons for this observation, one physiological and the other related to study design. For each subject, three or four serum samples were collected, while only one effect site sample was collected. Because of this limitation in study design, one might expect somewhat more variability in effect site parameter estimates. However, such variability is likely modest compared with the variability introduced by interindividual differences in pathophysiology, surgical interventions, and surgical trauma. Indeed, a study comparing the pharmacokinetics of gatifloxacin in plasma and sinus aspirate of patients with acute maxillary sinusitis showed a threefold-greater range of exposure in sinus aspirate compared with the range for plasma (1).

Effect site drug exposure has important implications as it helps explain unexpected clinical responses in patients infected with strains at the margins of susceptibility. The response to therapy is affected by multiple factors. For instance, the likelihood of a good clinical outcome is adversely affected when the patient has a high APACHE II score. A number of other covariates have an impact on the probability of a good clinical outcome. Two of the most important are the MIC of the pathogen being treated for the drug in question and the drug exposure at the primary effect site.

Using a set of isogenic strains of *Pseudomonas aeruginosa* with three different MICs for a fluoroquinolone, Drusano et al. were able to demonstrate that as the MIC increased, treatment with the same drug exposure resulted in increasing mortality (7). When the dose was increased so that an identical AUC-to-MIC ratio was attained in each animal cohort, however, survivorship levels were identical, regardless of the MIC of the infecting strain. This result is pertinent to the alteration in the variability of penetration to the infection site in patients with intra-abdominal infection. As discussed earlier, the penetration will likely be greater in infected patients. Thus, for patients infected with strains with higher MICs for tigecycline, it is likely that drug exposure at the infection site will be sufficient to attain an AUC-to-MIC ratio that will provide a high probability of a good clinical outcome.

In conclusion, we examined the penetration of tigecycline into colon wall tissue in subjects without infection and the penetration of tigecycline into ELF in subjects without infection. We found that penetration into colon wall tissue and ELF was greater than that in serum. Finally, we utilized Monte Carlo simulation to examine the expected range of tissue penetration. These simulations provided a description of individual variability in effect site exposure, the magnitude of which may help explain the clinical effectiveness of tigecycline against strains at the upper margin of susceptibility.

REFERENCES

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