In vivo Pharmacodynamic Evaluation of Omadacycline (PTK 0796) against Streptococcus pneumoniae in the Murine Pneumonia Model

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Running Title:
PK/PD of Omadacycline in the Murine Pneumonia Model

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ABSTRACT

Omadacycline is a novel aminomethylcyccline antibiotic in clinical development for community acquired bacterial pneumonia (CABP). We used a neutropenic murine pneumonia infection model to characterize the \textit{in vivo} pharmacodynamic activity of omadacycline against \textit{Streptococcus pneumoniae}. Four strains with varying phenotypic resistance to other antimicrobials including tetracyclines were utilized. Drug concentration measurements were performed in the plasma and epithelial lining fluid (ELF) after administration of 0.5, 2, 8, and 32 mg/kg. Pharmacokinetic parameters were calculated using a noncompartmental model and were linear over the dose range. Penetration into ELF ranged from 72-102%. Omadacycline demonstrated net cidal activity in relation to the initial burden against all 4 strains. The PK/PD index AUC/MIC correlated well with efficacy (R$^2$ 0.74). The plasma 24 h static dose AUC/MIC values were 16 – 20 (24 h ELF AUC/MIC of 14 – 18). A 1 log$_{10}$ kill was achieved at 24 h plasma AUC/MIC values of 6.1 – 180 (24 h ELF AUC/MIC values 6.0 – 200). A 2 log$_{10}$ kill was achieved at 24 h plasma AUC/MIC values of 19 – 56 (24 h ELF AUC/MIC of 17 – 47). The targets identified in this study in combination with \textit{in vitro} potency and favorable human pharmacokinetics make omadacycline an attractive candidate for further development and study in patients with CABP.
Lower respiratory tract infections are the second leading cause of morbidity and mortality worldwide (1). Despite vaccination efforts, *Streptococcus pneumoniae* continues to be the most common pathogen of community acquired bacterial pneumonia (CABP) irrespective of age and geographical location (2-4). Morbidity and mortality remain unacceptably high in part due to increasing drug resistance and limited effective antimicrobial options. Indeed, macrolide-resistance in *S. pneumoniae* has soared in recent years making it ineffective for many cases of *S. pneumonia* CABP (5, 6). Additionally, fluoroquinolones have recently undergone increased scrutiny regarding the potential risks of side effects or adverse effects during use (7, 8). Therefore, novel antibiotics effective against *S. pneumoniae* are urgently needed.

Omadacycline is a novel aminomethylcycline antibiotic in clinical development for CABP and skin and skin structure infections (9). The goals of our experiments were to characterize the *in vivo* pharmacokinetic/pharmacodynamic (PK/PD) properties of omadacycline. Specifically, we sought to determine [1] the serum and epithelial lining fluid (ELF) pharmacokinetics of omadacycline in the murine model and [2] the magnitude of the PK/PD parameter AUC/MIC required for efficacy against *S. pneumoniae* in the murine neutropenic pneumonia model.

**MATERIALS AND METHODS**

**Organisms, media, and antibiotic.** Four *S. pneumoniae* strains were used in the studies and are listed in Table 1. Unless specified by ATCC labelling, the strains are clinical isolates from invasive infections. Strains were chosen that varied in phenotypic resistance patterns to a number of relevant antimicrobials including penicillin,
minocycline, tigecycline and erythromycin. All organisms were grown, subcultured, and quantified using sheep blood agar (Remel, Milwaukee, WI). Drug compound for \textit{in vitro} and \textit{in vivo} studies were supplied by Paratek Pharmaceuticals (Boston, MA).

\textbf{In vitro susceptibility studies.} The minimum inhibitory concentrations (MICs) of each compound for the various strains were determined using Clinical and Laboratory Standards Institute (CLSI) microdilution methods (10). All MIC assays were performed in duplicate on three separate occasions. The median MIC of replicate assays is reported and utilized in PK/PD analyses.

\textbf{Murine model.} Animals were maintained in accordance with the American Association for Accreditation of Laboratory Animal Care (AAALAC) criteria (11). All animal studies were approved by the Animal Research Committees of the William S. Middleton Memorial VA Hospital and the University of Wisconsin. Six week-old, specific pathogen-free, female ICR/Swiss mice weighing 24 to 27 g were used for all studies (Harlan Sprague-Dawley, Indianapolis, IN). Mice were rendered neutropenic (neutrophils < 100/mm3) by injecting cyclophosphamide (Mead Johnson Pharmaceuticals, Evansville, IN) intraperitoneally 4 days (150 mg/kg) and 1 day (100 mg/kg) before lung infection. \textit{S. pneumoniae} strains were grown overnight on sheep blood agar. A sterile loop was then used to transfer organism to sterile saline and absorbance adjusted to 0.3 at 580 nm using a Spectronic 88 spectrophotometer (Bausch and Lomb, Rochester, NY). After a 1:10 dilution, bacterial counts of the inoculum ranged from $10^{3.4-8.1}$ CFU/mL. Lung infections with each of the strains were produced by administration of 50 μl of inoculum
into the nares of isofluorane-anesthetized mice. Mice were then held upright to allow for aspiration into the lungs. Therapy with omadacycline was initiated 2 h after induction of infection. No treatment controls and zero-hour controls were included in all experiments. After 24 h, organism burden was quantified by colony forming unit (CFU) counts from whole-organ homogenates.

Drug pharmacokinetics. Single dose plasma pharmacokinetics of omadacycline were performed in mice. Dose levels of 0.5, 2, 8, and 32 mg/kg were administered subcutaneously. Groups of three mice were sampled for drug concentration determination at 1, 2, 4, 6, 8, 12, and 24 hours. Both plasma and bronchial alveolar lavage (BAL) fluid was obtained for pharmacokinetic analysis. Plasma was obtained from each animal by centrifugation of anticoagulated blood obtained by cardiac puncture. BAL fluid was obtained by instillation of 1 ml of sterile saline into the lungs of each animal followed by immediate removal. The BAL fluid was centrifuged to remove blood and cellular debris, and supernatant was collected. Plasma and BAL supernatant was stored at -70°C. All drug concentrations were determined by LC-MS/MS methods by the sponsor. ELF concentrations were calculated from BAL concentrations by urea correction methodology (12) using the formula: [Drug]_{ELF} = [Drug]_{BAL} \times \frac{([Urea]_{plasma}/[Urea]_{BAL})}{\text{Urea correction}}.

Pharmacokinetic parameters (mean ± standard deviation), including elimination half-life (t_{1/2}), AUC_{0-\infty}, and Cmax, were calculated using a noncompartmental model using mean concentration values from each group of mice. The half-life was determined by linear least-squares regression. The AUC was calculated from the mean
concentrations using the trapezoidal rule. Pharmacokinetic estimates for dose levels that were not directly measured were calculated using linear interpolation for dose levels between those with measured kinetics and linear extrapolation for dose levels above or below the highest and lowest dose levels with kinetic measurements. Protein binding of omadacycline is very low and therefore total drug concentrations were utilized in all PK/PD calculations (13).

**Relationship between PK/PD parameter AUC/MIC and efficacy.** AUC/MIC was chosen as the pharmacodynamic parameter for omadacycline based on previous studies demonstrating this PK/PD index to be predictive of treatment efficacy for the tetracycline class (14-16). *In vivo* treatment studies were performed in the murine pneumonia model for each strain. Groups of three mice per dosing regimen and control group were utilized. Dose-response studies consisted of four-fold increasing doses (range 0.1-25.6 mg/kg/12 h) administered by subcutaneous route. The dose-response effect was determined as described above by measurement of CFUs in lung homogenates. The correlation between efficacy and the PK/PD parameter AUC/MIC was determined by nonlinear least-squares multivariate regression (SigmaPlot version 12.3; Systat Software, San Jose, CA). The mathematical model used was derived from the Hill equation \( E = \left( E_{max} \times \frac{AUC/MIC}{ED_{50} - AUC/MIC} \right)^N \), where \( E \) is the effector, in this case, the log change in CFU per lung between treated mice and untreated controls after the 24-h period of study, \( E_{max} \) is the maximum effect, \( D \) is the 24-h total AUC/MIC, \( ED_{50} \) is the AUC/MIC required to achieve 50% of the \( E_{max} \), and \( N \) is the slope of the dose-effect curve. The values for the indices \( E_{max} \), \( ED_{50} \), and \( N \) were calculated using...
nonlinear least-squares regression. The coefficient of determination ($R^2$) was used to estimate the variance that might be due to regression with the PK/PD parameter AUC/MIC.

**AUC/MIC magnitude associated with stasis and kill endpoints.** Using the Sigmoid Emax model described above, the dose required to produce net static effect (Static Dose), 1-, and 2- log$_{10}$ kill compared to the start of therapy was calculated for each drug-organism combination. The plasma and ELF pharmacokinetic results were then used to estimate the AUC/MIC exposure associated with each of the endpoints for each organism. The associated 24 h total drug AUC/MIC targets were calculated.

**RESULTS**

**In vitro susceptibility studies.** The MICs of omadacycline for the selected strains are listed in Table 1. Also shown are MICs to other relevant antimicrobial agents (when known). The four organisms varied in MIC to omadacycline by only four-fold despite phenotypic variation in susceptibility to other antimicrobials such as minocycline and tigecycline.

**Pharmacokinetics.** The time course of plasma concentrations of omadacycline in mice following subcutaneous doses of 0.5, 2, 8, and 32 mg/kg are shown in Figure 1. Over the dose range, the pharmacokinetics were relatively linear ($AUC R^2 = 1.00$, $C_{max} R^2 = 0.97$). Peak levels ranged from 0.11 to 2.19 mg/L. $AUC_{0-\infty}$ values ranged from 0.58 to 23.12 mg*h/L. The elimination half-life ranged from 2.8 to 6.3 hours.
ELF concentrations were determined from BAL concentrations utilizing the urea correction methodology described in methods above. Pharmacokinetic analysis of the data is presented in Figure 2. Over the dose range once again the pharmacokinetics were linear (AUC $R^2 = 0.99$, Cmax $R^2 = 0.99$). Peak levels ranged from 0.10 to 3.65 mg/L. AUC$_{0-\infty}$ values ranged from 0.47 to 16.76 mg*h/L. The elimination half-life ranged from 2.4 to 3.9 hours. The penetration of omadacycline into ELF relative to plasma drug concentrations was evaluated for each dose. Over the dose range, the percent penetration based on AUC exposure was 80% at 0.5 mg/kg, 94% at 2 mg/kg, 102% at 8 mg/kg, and 72% at 32 mg/kg.

**Relationship between PK/PD parameter AUC/MIC and efficacy.** At the start of therapy mice had $10^{6.3 \pm 0.3} \log_{10}$ CFU/lungs and this increased to $10^{7.8 \pm 0.6} \log_{10}$ CFU/lungs. The growth in untreated controls for each strain are shown in Table 2. The *in vivo* dose response curves for all four strains is shown in Figure 3. Omadacycline was quite potent over the dose range studied. Bactericidal activity was noted at all doses for two strains (140 and ATCC 49619) and 1 log-kill was achieved for all 4 strains over the dose range. A 3-log or greater kill was achieved with 3 of 4 strains. The relationship between log$_{10}$ CFU in lungs and 24 h plasma AUC/MIC ratio are illustrated in Figure 4. The relationship between plasma 24 h AUC/MIC and treatment effect was relatively robust with $R^2$ of 0.74. Also shown in the figure is the maximum effect (Emax), 50% maximal effect point (ED50), and the slope (N) of the best fit line based on the sigmoid (Hill) Emax model. The same PK/PD analysis for 24 h AUC/MIC is shown in Figure 5 using ELF pharmacokinetic data.
AUC/MIC magnitude associated with stasis and kill endpoints. The doses necessary to produce a bacteriostatic effect, 1-log and 2-log kill are shown in Table 2. The corresponding 24 h AUC/MIC for these doses are also presented utilizing both plasma and ELF pharmacokinetic data. The static doses were 0.92 mg/kg/24h and 1.28 mg/kg/24h against the two strains of S. pneumoniae for which this endpoint was achieved. The corresponding plasma 24 h AUC/MIC values were 16 and 20. The corresponding ELF 24 h AUC/MIC values were 14 and 18. A 1 log$_{10}$ kill was achieved for all S. pneumoniae strains with 24 h dose range of 0.45 - 18.2 mg/kg. The corresponding plasma and ELF AUC/MIC values were 6.1 - 180 and 6.0 - 200, respectively. The relatively larger range for 1 log kill targets was driven by a single isolate (1293) in which the target increased to a much larger degree when comparing stasis and 1 log kill AUC/MIC targets. The reason for this is not completely clear but could be due to inherent variability in the model and/or strain variability. Future studies utilizing more isolates would test whether this is an uncommon outlier. A 2 log$_{10}$ kill was achieved for three strains with a 24 h dose range of 1.8 - 3.1 mg/kg. The corresponding plasma and ELF 24 h AUC/MIC values were 19 - 56 and 17 – 47, respectively. The AUC/MIC values are relatively similar given the relative penetration of drug into ELF ranged from 72-102% over the dose range.

DISCUSSION

The tetracycline class of antibiotics has been around for over 70 years; however, there has been growing antibacterial resistance, especially in recent years. Advances
in synthetic chemistry has created renewed interest in derivatives of tetracycline that maintain antimicrobial activity despite acquired tetracycline resistance mechanisms such as efflux pumps and ribosomal protection. This has been successfully applied in the generation of glycylnyclines, fluorocyclines, and the aminomethylcyclines, such as omadacycline which was the focus of these studies. Omadacycline is a novel, first-in class aminomethylcycline antibiotic in development for the treatment of CABP and skin and skin structure infection. Omadacycline is a broad-spectrum agent with potency against a variety of pathogens including gram-positives (including those with resistance to beta-lactams such as MRSA and to macrolides like \textit{S. pneumoniae}), gram-negatives, anaerobes and atypical pathogens (9, 17, 18). Importantly, omadacycline maintains excellent activity against organisms that have acquired resistance to older tetracyclines (tetracycline, minocycline and doxycycline) (19, 20).

Pharmacodynamic assessment of antimicrobial efficacy is a critical step in drug development to determine the optimal dosing strategy for clinical studies as well as set preliminary susceptibility breakpoints. In this manuscript, we present the results of a pharmacodynamic assessment of omadacycline activity in a preclinical animal model of \textit{S. pneumoniae} pneumonia. Omadacycline demonstrated \textit{in vitro} and \textit{in vivo} potency against a select group of \textit{S. pneumoniae} strains including those with resistance to other antibiotics such as beta-lactams, macrolides, and earlier generations of the tetracycline class. \textit{In vivo} we observed potent bactericidal activity for all 4 strains with a $\geq 3 \log_{10}$ kill in 3 of 4 strains tested. We also examined the drug exposures associated with efficacy in terms of plasma pharmacokinetic and ELF pharmacokinetic exposures. Previous unpublished data had suggested favorable pharmacokinetics in lung penetration and we
affirmed these preliminary results. Almost 100% of the drug in plasma penetrated into
the ELF compartment based on drug concentration measurements in both
compartments. This translated into similar PK/PD targets when one compared plasma
to ELF AUC/MIC targets for stasis or bactericidal endpoints. Additionally, the exposure-
response curves were quite steep such that small increases in drug exposure led to
increasing cidal activity.

There is a paucity of pharmacodynamic studies for the tetracycline class,
including new synthetic congeners, to provide a comparison to the current studies.
Previous studies have demonstrated tetracyclines exhibit time dependent activity with
prolonged post-antibiotics effects (14-16). Therefore, the predictive PK/PD index has
often been found to be AUC/MIC. This has been demonstrated in dose-fractionation
studies with two other synthetic tetracycline derivatives tigecycline (16) and
eravacycline (unpublished, submitted for publication to AAC Oct, 2016).
Pharmacodynamic studies evaluating the PK/PD target exposures in in vivo animal
model studies are even more sparse. Utilizing 4 S. pneumoniae strains in a murine
neutropenic thigh model, Christianson and colleagues demonstrated a 24 h free drug
AUC/MIC target of 24 for doxycycline was associated with net stasis and a value of 120
was associated with a 2 log_{10} kill (21). Tigecycline PK/PD studies have also
demonstrated the importance of AUC/MIC as the PK/PD driver of efficacy with net
stasis targets that have ranged from 2-5 for a variety of pathogens (22-24). The
AUC/MIC exposures associated with efficacy in the current pre-clinical model for
omadacycline are similar to other tetracycline class antibiotic PK/PD studies.
Importantly, in the case of tigecycline, clinical PK/PD analyses in patients with skin and
skin structure infection and community-acquired pneumonia have confirmed the relevance of the PK/PD targets identified in pre-clinical models on treatment outcome (25, 26).

Human pharmacokinetic studies have evaluated the plasma pharmacokinetics of omadacycline after 100 mg IV and 300 mg oral tablet administration (18). The AUC_{0-inf} is nearly identical at 10.0 and 10.3 mg*h/L, respectively. In 2014, surveillance antimicrobial susceptibility results for omadacycline against *S. pneumoniae* (over 1800 strains) demonstrated a MIC_{90} of 0.06 mg/L (range of 0.015 – 0.12 mg/L) (18). Likewise, in 2015 the *in vitro* activity of omadacycline was examined against tetracycline-resistant strains and demonstrated a MIC_{90} of 0.25 mg/L (range 0.015 – 0.25) (18). Utilizing the median AUC/MIC drug exposure targets identified in this study for stasis and kill endpoints, the AUC exposures in humans using the above dosing regimens would be expected to produce efficacy against most *S. pneumoniae* strains, including those with tetracycline resistance. Given the favorable PK, including low protein binding and penetration into ELF, *in vitro* potency that included tetracycline-resistant *S. pneumoniae* strains, and *in vivo* efficacy observed in our animal model study, omadacycline is a promising novel agent for community acquired pneumonia due to *S. pneumoniae*. These studies should prove beneficial in optimizing clinical dosing regimen design for CABP and setting preliminary susceptibility breakpoints.

**ACKNOWLEDGMENTS**

This study was funded by Paratek Pharmaceuticals.
REFERENCES


Table 1. Study organisms and omadacycline susceptibility results. Where known, the antimicrobial susceptibility results to other relevant antimicrobial agents are also listed in the table.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Omadacycline MIC (mg/L)</th>
<th>Tigecycline MIC (mg/L)</th>
<th>Minocycline MIC (mg/L)</th>
<th>Erythromycin MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. pneumoniae 1293&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0625</td>
<td>0.06</td>
<td>4</td>
<td>&gt;8 (Erm)</td>
</tr>
<tr>
<td>S. pneumoniae ATCC 10813&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0625</td>
<td>0.12</td>
<td>0.25</td>
<td>0.015</td>
</tr>
<tr>
<td>S. pneumoniae 140&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.125</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>S. pneumoniae ATCC 49619&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03125</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

<sup>a</sup> Penicillin-Resistant
<sup>b</sup> Penicillin-Susceptible
<sup>c</sup> NA, Not available
Table 2. 24 h static, 1 log, and 2 log kill doses and associated AUC/MIC values for each strain in the murine pneumonia model.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Stasis</th>
<th>1 log&lt;sub&gt;10&lt;/sub&gt; kill</th>
<th>2 log&lt;sub&gt;10&lt;/sub&gt; kill</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h Growth in Untreated control animals (log&lt;sub&gt;10&lt;/sub&gt; CFU/lungs)</td>
<td>MIC (mg/L)</td>
<td>24 h total dose (mg/kg)</td>
</tr>
<tr>
<td>1293</td>
<td>2.34</td>
<td>0.06</td>
<td>1.28</td>
</tr>
<tr>
<td>10813</td>
<td>1.64</td>
<td>0.06</td>
<td>0.92</td>
</tr>
<tr>
<td>140</td>
<td>1.13</td>
<td>0.125</td>
<td>NA</td>
</tr>
<tr>
<td>49619</td>
<td>0.86</td>
<td>0.03</td>
<td>NA</td>
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</tbody>
</table>

NA, endpoint not achieved
Figure Legends

Figure 1. Plasma concentrations of omadacycline in mice following single subcutaneous doses. Samples were obtained at seven time points over 24 hours. Each symbol represents the mean and standard deviation from three mice. Cmax represents the peak concentration, T1/2 the beta elimination half-life, and AUC is from 0 to infinity.

Figure 2. ELF concentrations of omadacycline in mice following single subcutaneous doses. Samples were obtained at seven time points over 24 hours. ELF concentrations were determined using urea concentration correction methods. Each symbol represents the mean and standard deviation from three mice. Cmax represents the peak concentration, T1/2 the beta elimination half-life, and AUC is from 0 to infinity.

Figure 3. In vivo dose-response curves for omadacycline against select S. pneumoniae strains using a neutropenic murine pneumonia model. Each symbol represents the mean and standard deviation from three mice. Five total drug dose levels were administered by subcutaneous route every 12 hours. The burden of organisms was measured at the start and end of therapy. The study period was 24 hours. The horizontal dashed-line at 0 represents the burden of organisms in the lungs of mice at the start of therapy. Data points below the line represent cidal activity and points above the line represent net growth.

Figure 4. In vivo dose effect of omadacycline against select S. pneumoniae strains using a neutropenic murine pneumonia model. Each symbol represents the mean result from three mice. Five total drug dose levels were fractionated into an every 12 hourly regimen. The omadacycline exposure is expressed as the plasma 24h AUC/MIC. The burden of organisms was measured at the start and end of therapy. The study period was 24 hours. The horizontal dashed-line at 0 represents the burden of organisms in the lungs of mice at the start of therapy. Data points below the line represent cidal activity and points above the line represent net growth. The R² represents the coefficient of determination. The line drawn through the data points is the best fit line based upon the sigmoid Emax formula.

Figure 5. In vivo dose effect of omadacycline against select S. pneumoniae strains using a neutropenic murine pneumonia model. Each symbol represents the mean result from three mice. Five total drug dose levels were fractionated into an every 12 hourly regimen. The omadacycline exposure is expressed as the ELF 24h AUC/MIC. The burden of organisms was measured at the start and end of therapy. The study period was 24 hours. The horizontal dashed-line at 0 represents the burden of organisms in the lungs of mice at the start of therapy. Data points below the line represent cidal activity and points above the line represent net growth. The R² represents the coefficient of determination. The line drawn through the data points is the best fit line based upon the sigmoid Emax formula.