# *In vivo* Pharmacodynamic Evaluation of Omadacycline (PTK 0796) against *Staphylococcus aureus* (SA) in the Murine Thigh Infection Model

A. J. Lepak[1], M. Zhao[1], K. Marchillo[2], J. VanHecker[2], D. R. Andes[1]

[1]University of Wisconsin School of Medicine and Public Health, Madison, WI; [2]William S. Middleton Memorial Veterans Hospital, Madison, WI

1531

Contact Information:
Alexander J. Lepak, MD
University of Wisconsin
Madison, WI 53705
Phone: (608) 263-1545
Fax: (608) 263-4464
ajlepak@medicine.wisc.edu

## ABSTRACT

**Background:** Omadacycline is a novel aminomethylcycline antibiotic in development for acute bacterial skin and skin structure infection (ABSSSI) and community acquired bacterial pneumonia (CABP). The goal of the study was to determine the PK/PD targets in the murine thigh infection model against a diverse group of SA pathogens including MRSA.

**Methods:** 10 SA strains (4 MSSA, 6 MRSA) were utilized. MICs were determined using CLSI methods. Single dose murine plasma PK was previously determined in our lab and used for PK/PD analyses. The neutropenic murine thigh infection model was utilized for all treatment studies and drug dosing was by subcutaneous route. Four-fold increasing doses of omadacycline (0.25-64 mg/kg) were administered q12h to groups of mice infected with each strain. Treatment outcome was measured by determining organism burden in the thighs (CFU) at the end of each experiment (24 h). The Emax Hill equation was used to model the dose-response data to the PK/PD index AUC/MIC. The magnitude of the PK/PD index AUC/MIC associated with net stasis and 1-log kill were determined in the thigh model for all strains.

**Results:** MICs ranged from 0.25-0.5 mg/L. At the start of therapy, mice had 7.1 ± 0.3 $\log_{10}$ CFU/thigh. In control mice, the organism burden increased 2.3 ± 0.3 $\log_{10}$ CFU/thigh over 24 h. There was a relatively steep dose-response relationship observed with escalating doses of omadacycline. Maximal organism reductions were 4-5 $\log_{10}$ CFU/thigh compared to untreated controls. Stasis and 1 log-kill (from start of therapy) was observed against each strain. The AUC/MIC magnitude associated with stasis and 1-log kill endpoints are shown in the table.

| SA Group (n=10) | 24 h Static Dose (mg/kg) | Stasis AUC/MIC | 24 h 1 log kill Dose (mg/kg) | 1 log kill AUC/MIC |
|---|---|---|---|---|
| Mean | 13.9 | 23.7 | 45.7 | 78.1 |
| Median | 13.0 | 21.9 | 39.8 | 57.7 |
| Std Dev | 4.3 | 10.6 | 31.4 | 79.5 |

**Conclusion:** Omadacycline demonstrated in vivo potency against a diverse group of SA pathogens including MRSA strains. Stasis 24 h AUC/MIC targets were approximately 24. This is very similar to previous studies of omadacycline against *S. pneumoniae* (stasis AUC/MIC 18) and other PK/PD evaluations of tetracycline-class antibiotics. 1-log kill targets were only 2-3 fold more than stasis targets for each strain. This data should provide useful in the dose-regimen optimization of omadacycline.

## BACKGROUND

- Omadacycline (PTK 0796) is a novel aminomethylcycline antibiotic in clinical development for acute bacterial skin and skin structure infection (ABSSSI) and community acquired bacterial pneumonia (CABP)
- Previous studies have demonstrated AUC/MIC as the pharmacodynamic driver of efficacy for tetracycline-based antimicrobial agents
- A previous PK/PD study using the murine pneumonia model demonstrated efficacy against *S. pneumoniae* with relatively low AUC/MIC exposures of 15-20 associated with net stasis
- This study was designed to add to the pre-existing PK/PD data by studying the PD exposures associated with stasis and cidal endpoints in the neutropenic murine thigh model against a diverse groups of SA strains

## METHODS

Strains and susceptibility testing: 10 *S. aureus* strains were utilized including ATCC and clinical isolates as well as 6 MRSA and 4 MSSA (see Table 1). All isolates were tested in accordance with CLSI methodology. MICs were performed on three separate occasions in duplicate.

Omadacycline Pharmacokinetics: Pharmacokinetic studies in mice were performed and published previously by our group (1). The pharmacokinetic data from this study was used to model AUC/MIC exposures. Protein binding of omadacycline is negligible and therefore total drug concentrations were utilized in all calculations.

Murine Thigh Infection Model: Mice were rendered neutropenic by cyclophosphamide injection. Broth cultures were grown overnight to log phase to produce an inoculum of 6.5 ± 0.09 $\log_{10}$ CFU/ml. Thigh infections were produced by injection of 0.1 ml of the inoculum into the thighs of isoflurane-anesthetized mice 2 h before treatment. Organism burden (CFU) was determined at the start of therapy and after 24 h from thigh homogenates. Four thigh infections were included per treatment and control group.

Treatment efficacy - pharmacodynamic target determination: Drug dosing consisted of 0.25, 1, 4, 16, and 64 mg/kg of omadacycline every 12 h by SC administration. At the start of therapy, mice had 7.1 ± 0.4 $\log_{10}$ CFU/thigh and grew to 9.4 ± 0.4 $\log_{10}$ CFU/thigh in untreated controls. The treatment results were analyzed using a sigmoid maximum effect (Emax) model. The doses corresponding with net stasis and 1-log kill for each strain were calculated using the Hill Emax equation. The total drug AUC/MIC associated with each endpoint were calculated for each strain.

1. Lepak AJ, et al. 2017. Antimicrob Agents Chemother 61(5). pii: e02368-16. doi: 10.1128/AAC.02368-16

## RESULTS

**Figure 1.** Dose-response curves for 10 SA strains against omadacycline in the neutropenic murine thigh model. Each data point is the mean (standard deviation) of four thigh replicates. The horizontal dashed line is the burden of organisms at the start of therapy. Points above the line represent net growth while those below represent net cidal activity. Blue data points/lines = MSSA and Red data points/lines = MRSA

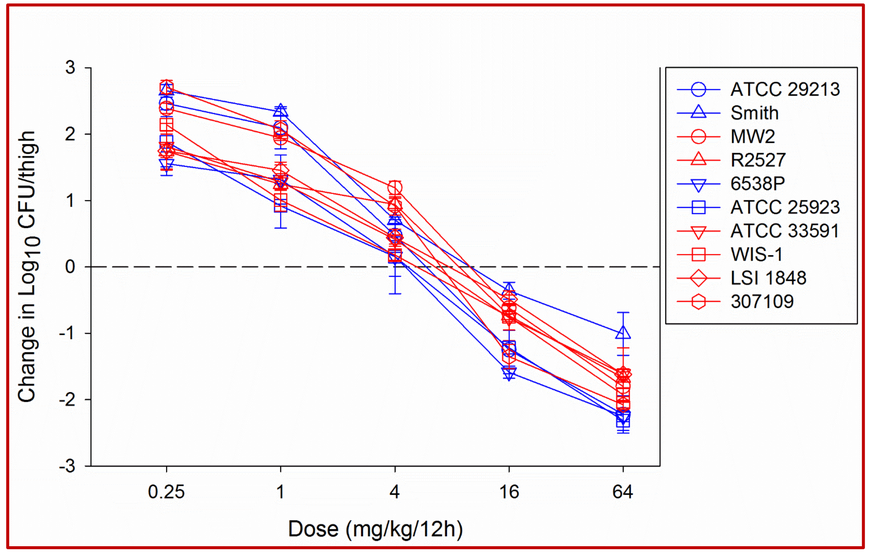


**Figure 2.** *In vivo* pharmacodynamic modelling of the PK/PD index AUC/MIC and treatment effect in the neutropenic murine thigh infection model against 10 SA strains. Each data point is the mean (standard deviation) of four thigh replicates. The horizontal dashed line is the burden of organisms at the start of therapy. Points above the line represent net growth while those below represent net cidal activity. The curve is the best fit line based on the Hill equation. Also shown are the PD parameters Emax (maximal effect), ED50 (half maximal effect), slope (N), and the coefficient of determination ($R^2$).

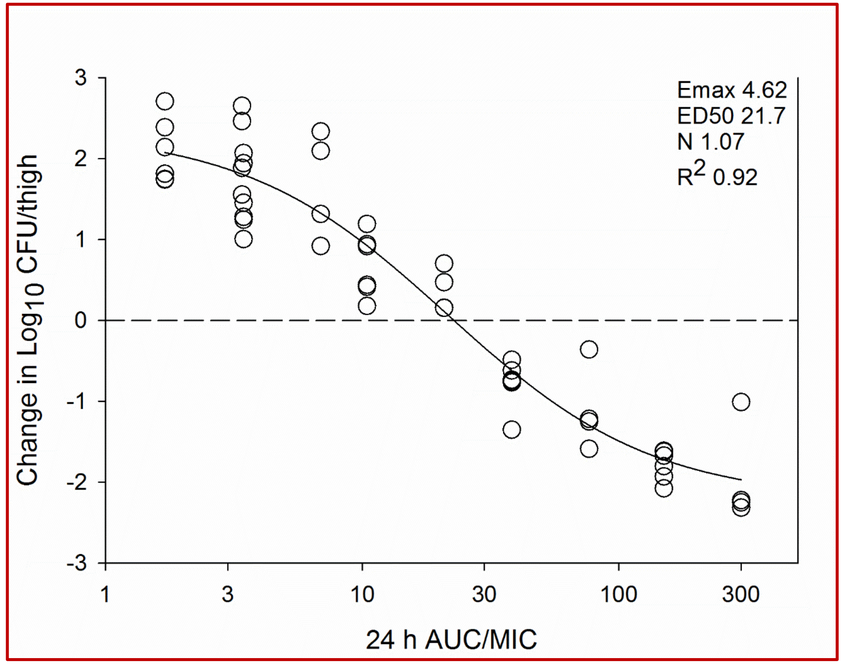


**Table 1. Select Strains used in study and In vitro Susceptibility Results:**

| SA Strain | Omadacycline MIC (mg/L) | Phenotype |
|---|---|---|
| 307109 | 0.5 | MRSA |
| LSI 1848 | 0.5 | MRSA |
| WIS-1 | 0.5 | MRSA |
| ATCC 33591 | 0.5 | MRSA |
| ATCC 25923 | 0.25 | MSSA |
| ATCC 29213 | 0.25 | MSSA |
| SMITH | 0.25 | MSSA |
| MW2 | 0.5 | MRSA |
| R2527 | 0.5 | MRSA |
| 6538P | 0.25 | MSSA |

**Table 2. PK/PD target exposures associated with net stasis and 1-log kill**

| Organism | Growth in Untreated Controls ($\log_{10}$ CFU) | 24 h Static Dose (mg/kg) | 24 h Stasis AUC/MIC | 24 h 1-log kill Dose (mg/kg) | 1-log kill AUC/MIC |
|---|---|---|---|---|---|
| ATCC 29213 | 2.63 | 11.67 | 29.64 | 24.20 | 58.83 |
| SMITH | 2.78 | 20.88 | 51.13 | 128.00 | 302.51 |
| MW2 | 2.49 | 18.78 | 23.12 | 44.07 | 52.49 |
| R2527 | 2.05 | 17.54 | 21.68 | 52.32 | 62.06 |
| 6538P | 1.96 | 8.43 | 22.05 | 19.94 | 48.95 |
| ATCC 25923 | 2.22 | 8.72 | 22.71 | 25.40 | 61.63 |
| ATCC 33591 | 2.47 | 12.84 | 16.19 | 47.62 | 56.61 |
| WIS-1 | 2.31 | 10.80 | 13.80 | 35.45 | 42.48 |
| LSI 1848 | 1.89 | 16.44 | 20.41 | 53.00 | 62.86 |
| 307109 | 2.84 | 13.12 | 16.52 | 26.57 | 32.17 |
| **Mean** | | **13.92** | **23.73** | **45.66** | **78.06** |
| **Median** | | **12.98** | **21.87** | **39.76** | **57.72** |
| **Std Dev** | | **4.28** | **10.61** | **31.42** | **79.47** |

## CONCLUSION

**1]** Omadacycline demonstrated potent *in vitro* and *in vivo* activity against a diverse group of MSSA and MRSA clinical strains

**2]** The exposure-response relationship was well described by AUC/MIC ($R^2$ 0.92) and was relatively steep with >1 log kill achieved against every strain

**3]** The median stasis AUC/MIC target was 22 and median 1-$\log_{10}$ kill AUC/MIC target was 58. These values are similar to previous study of omadacycline against *S. pneumoniae* as well as previous studies with other tetracycline derivatives.

**4]** Interestingly, there was evidence of enhanced efficacy against MRSA strains in this study as the median values were significantly different for stasis (MRSA stasis AUC/MIC of 18, MSSA stasis AUC/MIC of 26, $p$=0.038 by Mann-Whitney Rank Sum). These differences were smaller and not significant for median 1-$\log_{10}$ kill targets (MRSA 1-$\log_{10}$ kill AUC/MIC of 55, MSSA 1-$\log_{10}$ kill AUC/MIC of 60, $p$=0.48).

**5]** These studies should prove very useful in continued clinical development and optimization of omadacycline for *S. aureus* infections including designing optimal dosing strategies as well as preliminary breakpoints

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