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EXPERIMENTAL THERAPEUTICS

In Vivo Pharmacodynamics of Omadacycline against *Staphylococcus aureus* in the Neutropenic Murine Thigh Infection Model

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ABSTRACT Omadacycline is a novel aminomethylcycline antibiotic with potent activity against *Staphylococcus aureus*, including methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA). We investigated the pharmacodynamic activity of omadacycline against 10 MSSA/MRSA strains in a neutropenic murine thigh model. The median 24-h area under the concentration-time curve (AUC)/MIC values associated with net stasis and 1-log kill were 21.9 and 57.7, respectively.

KEYWORDS *Staphylococcus aureus*, omadacycline, pharmacodynamics

Omadacycline (Nuzyra; Paratek Pharmaceuticals), an aminomethylcycline antibiotic within the tetracycline class, was approved in October 2018 in the United States for the treatment of adults with acute bacterial skin and skin structure infection (ABSSSI) and community-acquired bacterial pneumonia (CABP) based on results from three large randomized controlled trials (1, 2). Previous *in vitro* and *in vivo* studies have demonstrated potent Gram-positive activity for omadacycline that includes methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) (4–8). We have previously characterized the pharmacokinetics and pharmacodynamic activity of omadacycline against *Streptococcus pneumoniae* using a neutropenic murine pneumonia infection model (9). In the current studies, we explored the *in vivo* activity of omadacycline against multiple strains of *S. aureus*, including MRSA, to delineate its pharmacodynamic activity and target exposures for stasis and log cidal reduction in the murine thigh infection model.

Ten *S. aureus* strains were utilized (Table 1) that included 6 MRSA and 4 MSSA strains. MICs were determined in triplicate according to CLSI guidelines (10). The MIC range was very narrow, at 0.25 to 0.5 mg/liter. This has been demonstrated in previous studies, with MIC_{50}/MIC_{90} values that have only varied by a 2-fold dilution (6, 7, 11). The neutropenic murine thigh infection model was used for *in vivo* study of omadacycline. Animals were maintained in accordance with American Association for Accreditation of Laboratory Animal Care (AAALAC) criteria. All animal studies were approved by the Animal Research Committee of the William S. Middleton Memorial VA Hospital and the University of Wisconsin. Mice were infected with 6.5 ± 0.1 $\log_{10}$ CFU of each strain/thigh. The average *in vivo* fitness (growth in untreated control mice) of each strain was 2.3 ± 0.3 $\log_{10}$ CFU/thigh over 24 h (range, 1.89 to 2.78 $\log_{10}$ CFU). Two hours after thigh infection, omadacycline was administered to mice by the subcutaneous route every 12 h over the 24-h experiment duration. Omadacycline was administered according to one of five dosing regimens (dose range, 0.25 to 64 mg/kg of body weight/12 h in 4-fold increments). Net stasis and a 1-log reduction in CFU were observed over the dose range for every strain (Fig. 1). The dose-response curves were similar over the dose

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TABLE 1 *Staphylococcus aureus* strains and susceptibility results

Strain	Omadacycline MIC (mg/liter)	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

range, as anticipated, given the relative similarity of the MIC values for all strains. Efficacy was similar against MSSA and MRSA strains.

We assessed the pharmacodynamic relationship of the area under the concentration-time curve (AUC)/MIC, as this has been shown in multiple studies to be the most predictive index of therapeutic effect (12–14). Utilizing our previously characterized murine pharmacokinetics of omadacycline from this infection model (9), which demonstrated linear AUC pharmacokinetics ($R^2 > 0.99$), we were able to estimate total drug AUC exposures over the dose range. The resultant AUC/MIC exposures are represented in Fig. 2 for each strain and dosing regimen. There was a strong relationship between AUC/MIC and therapeutic effect, with an R^2 of 0.92 when modeled according to the sigmoid maximum effect (E_{max}) model (Hill equation). The AUC/MIC exposures associated with net stasis and 1-log kill for each strain are shown in Table 2. Stasis was demonstrated at a median 24-h AUC/MIC of approximately 22. One-log kill was noted at a median 24-h AUC/MIC of approximately 58. It should be noted only total drug concentrations were utilized in these studies, as the protein binding of omadacycline is relatively low (~20%), similar in humans and mice, and without evidence of concentration-dependent effect that has been noted with others in the tetracycline class (15).

These results add to our understanding of omadacycline exposure-response rela-

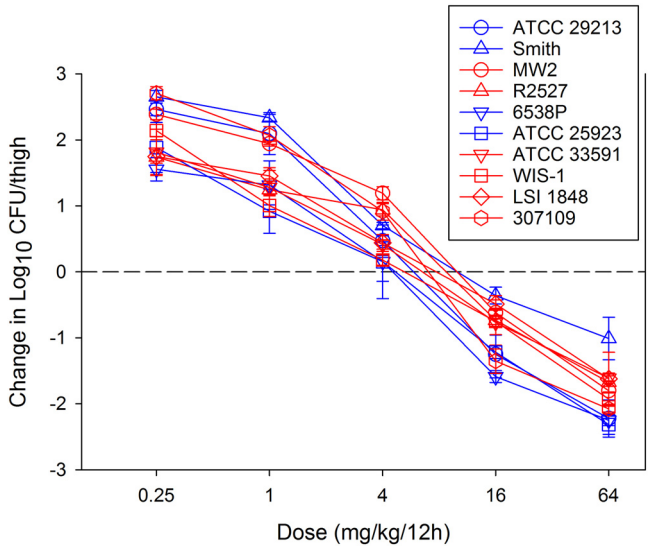

FIG 1 Dose-response curves for omadacycline against 10 *S. aureus* (blue symbols, MSSA; red symbols, MRSA) strains in the neutropenic murine thigh infection model. Each symbol represents the mean and standard deviation from four thigh infection replicates. Five different dose levels were administered by subcutaneous route every 12 h. The burden of organisms was enumerated at the start and end of therapy over a 24-h experiment duration. The horizontal dashed line at 0 represents the burden of organisms at the start of therapy. Data points above the line represent a net growth (i.e., increase) in burden, and those below the line represent net cidal activity (i.e., decrease) in bacterial burden.

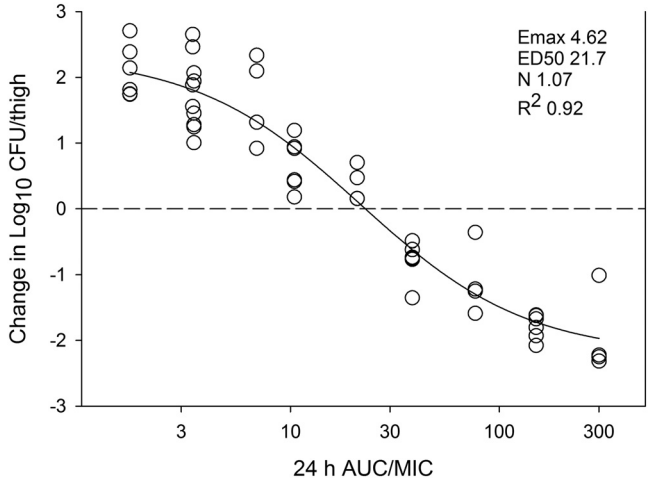

FIG 2 *In vivo* exposure-response relationship between the pharmacodynamic parameter 24-h AUC/MIC and treatment effect for 10 *S. aureus* strains in the neutropenic murine thigh infection model. Each symbol is the mean of four thigh replicates. Five total drug dosing regimens were fractionated into an every-12-h regimen. The omadacycline exposure is represented on the *x* axis as plasma 24-h AUC/MIC. The burden of organisms was measured at the start and end of therapy over a 24-h experiment duration. The change (i.e., difference) between the start and end of therapy is represented on the *y* axis. The horizontal dashed line at 0 represents the burden of organisms at the start of therapy. Data points above the line represent a net increase, and those below the line represent a net decrease in bacterial burden. The line drawn through the data is the best-fit line based on the sigmoid E_{max} model (Hill equation). Also shown are the pharmacodynamic parameters E_{max} (maximum effect), ED_{50} (50% maximal effect point), N (slope of the line), and coefficient of determination (R^2).

tionships. First, not unexpectedly, *in vivo* efficacy and pharmacodynamic target exposures were similar for MSSA and MRSA. This confirms *in vitro* evaluations that have previously demonstrated very similar potency with comparable MIC distributions for these two pathogen groups. Second, against all strains in this study, the exposure-response relationship was relatively steep, with >1-log kill achieved against all strains. The microbiological activity noted in this study helps explain the high rates of efficacy noted in patients with MSSA or MRSA infections in the omadacycline clinical trials (2, 16). Third, we demonstrated a strong relationship between AUC/MIC and therapeutic effect ($R^2 = 0.92$). Similar results have been shown for other agents in the tetracycline class (9, 12–14, 17, 18), confirming that the pharmacokinetic/pharmacodynamic (PK/PD) driver of efficacy for this class is the AUC/MIC. Finally, previous preclinical and clinical evaluations have demonstrated the predictive value of stasis endpoints in the murine model with clinical outcome for patients with bacterial skin and skin structure infections (14, 19). Large surveillance studies have demonstrated an estimated MIC_{90} of ≤0.25 mg/liter (11, 20). Integrating these data with human pharmacokinetic estimates

TABLE 2 Omadacycline pharmacodynamic target exposures for each *S. aureus* strain in the murine thigh infection model

Organism or measurement	MIC (mg/liter)	Growth in untreated controls (log_{10} CFU)	24-h static dose (mg/kg)	Stasis AUC/MIC	24-h 1-log kill dose (mg/kg)	1-log kill AUC/MIC
ATCC 29213	0.25	2.63	11.67	29.64	24.20	58.83
SMITH	0.25	2.78	20.88	51.13	128.00	302.51
MW2	0.5	2.49	18.78	23.12	44.07	52.49
R2527	0.5	2.05	17.54	21.68	52.32	62.06
6538P	0.25	1.96	8.43	22.05	19.94	48.95
ATCC 25923	0.25	2.22	8.72	22.71	25.40	61.63
ATCC 33591	0.5	2.47	12.84	16.19	47.62	56.61
WIS-1	0.5	2.31	10.80	13.80	35.45	42.48
LSI 1848	0.5	1.89	16.44	20.41	53.00	62.86
307109	0.5	2.84	13.12	16.52	26.57	32.17
Mean		2.33	13.92	23.73	45.66	78.06
Median		2.31	12.98	21.87	39.76	57.72
SD		0.34	4.28	10.61	31.42	79.47

(8), the stasis AUC/MIC target identified in this study would be exceeded in almost all patients.

In sum, these results suggest that omadacycline is a promising agent against *S. aureus*, including MRSA. The pharmacodynamic targets identified in the murine thigh model for net stasis suggest achievability for most patients with bacterial skin and skin structure infection when examining the targets in the context of human pharmacokinetics of approved dosing regimens and epidemiological MIC distribution. Future studies are warranted to examine the pharmacodynamic activity of omadacycline against *S. aureus* at other sites of infection, such as pneumonia.

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