

Sub-growth-inhibitory concentrations of omadacycline inhibit *Staphylococcus aureus* haemolytic activity *in vitro*

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Objectives: To evaluate the effect of sub-growth-inhibitory concentrations of omadacycline on *Staphylococcus aureus* ATCC 10832 haemolytic activity *in vitro*.

Methods: Following determination of the MICs of omadacycline and comparator antibiotics, the strain was grown in the presence of individual antibiotics and the percentage of haemolysis assayed; ‘washout’ experiments were performed with omadacycline only.

Results: Omadacycline inhibited *S. aureus* haemolytic activity *in vitro* at sub-growth-inhibitory concentrations. Inhibition was maintained at least 4 h after removal of extracellular drug.

Conclusions: Omadacycline’s *in vitro* potency and suppression of virulence factors might contribute to its efficacy in the treatment of acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia caused by virulent strains of *S. aureus*. This finding could be relevant for other organisms and virulence factors that depend on new protein synthesis.

Introduction

Certain bacteria produce virulence factors, which contribute to the ability of a microorganism to cause an infection, and the severity of an infection—e.g. causing tissue destruction by interfering with the host response to infection, or causing uncontrolled inflammation.^{1–3} Favourable treatment outcomes can be achieved by killing the infecting organism or by preventing its multiplication, and agents that can also prevent or inhibit virulence factor production and/or activity may offer additional benefit. In animal models of *Staphylococcus aureus* infection, α -haemolysin, a pore-forming exotoxin that can damage the host cell’s plasma membrane, has been shown to be a key virulence factor.^{1–4}

Virulence factor production largely depends on protein synthesis, and protein synthesis inhibitor antibiotics have been shown to suppress the production of proteinaceous exotoxins in organisms such as *S. aureus*.⁵ Indeed, the IDSA clinical practice guidelines for the treatment of MRSA infections state that protein synthesis inhibitor antibiotics such as clindamycin and linezolid may be considered as adjunctive therapy in some scenarios.⁶ Exposure to protein synthesis inhibitors at levels at or below concentrations required to inhibit bacterial multiplication may decrease α -haemolysin expression^{7–9} and could therefore provide continued antivirulence effects independent of the agent’s effect on organism growth inhibition. Therefore, the use of recommended doses of protein synthesis

inhibitors to treat infections caused by *S. aureus* could provide the necessary exposure to inhibit *S. aureus* multiplication, with the possible added benefit of continued inhibition of the production of *S. aureus*-associated virulence factors.

Omadacycline, a novel aminomethylcycline antibiotic derived from the tetracycline class of bacterial protein biosynthesis inhibitors, shows activity against methicillin-susceptible *S. aureus* (MSSA) and MRSA.¹⁰ It is approved in the United States for the treatment of adult patients with community-acquired bacterial pneumonia and acute bacterial skin and skin structure infections caused by susceptible microorganisms, including *S. aureus*.¹⁰ We sought to determine the durability of inhibition and effect of sub-growth-inhibitory concentrations of omadacycline on *S. aureus* haemolytic activity.

Materials and methods

All experiments were conducted with MSSA Wood 46 (ATCC 10832), which secretes high levels of α -haemolysin.¹¹ *S. aureus* cultures were grown in cation-adjusted Mueller Hinton II (MH) broth media (Becton Dickinson) and on tryptic soy agar plates with 5% sheep blood (Northeast Laboratory Services) at 37°C. MICs of omadacycline and comparator antibiotics were determined using CLSI methodology in cation-adjusted MH broth.¹² Comparators included tetracycline, clindamycin, linezolid, vancomycin, and cephalothin.

Growth of *S. aureus* with antibiotics

Antibiotics were added to broth cultures during mid-log growth. At 4 h after drug addition, cfu/mL were determined. Filtered supernatants were tested for haemolytic activity by incubation with rabbit erythrocytes. Control samples included drugs with media and no bacteria, to show that the drug alone does not cause haemolysis, and drugs added to fully grown cultures before filter sterilizing, to show that the drug was not interfering with detection of haemolytic activity.

In washout experiments, omadacycline was added to mid-log broth cultures for 1 h. Spent media, after centrifugation, was removed, and pelleted bacteria were resuspended in the same volume of fresh media without drug and incubated for another 4 h.

Haemolysis assay

Filtered culture supernatant aliquots were diluted and mixed with an equal volume of 2% defibrinated rabbit blood (Remel) in PBS in microtitre plates. MH broth was used as a control for 0% haemolysis; MH broth containing lysis solution [9% (v/v) solution of Triton-X-100 (Promega)] was used as a control for 100% haemolysis. Purified *S. aureus* α -haemolysin (Toxin Tech, HP101) was also included as a control (a concentration curve was constructed for each plate: 100 μ L of 1 mg/mL stock reagent was diluted in 900 μ L of MH broth with 1% DMSO; 500 μ L was then diluted with 500 μ L of MH broth with 1% DMSO, and this was repeated for a total of seven dilutions). After incubation at 37°C for 1 h, the microtitre plates were centrifuged and the supernatant transferred to another microtitre plate for analysis. The average absorbance at 450 nm ($A_{450\text{ nm}}$) of media-only wells was subtracted from the $A_{450\text{ nm}}$ of all other wells as background, recorded via a Victor V plate reader. Data were expressed as: % haemolysis = (sample $A_{450\text{ nm}}$ /average lysed $A_{450\text{ nm}}$) \times 100. The percentage haemolysis data for each dilution series were plotted with Excel, and XLfit was used to determine the volume of sample required to cause 50% haemolysis, which equals 1 haemolytic unit. Haemolytic units/mL culture were calculated and dilution plating results were used to convert data to haemolytic units/cfu.

Data availability

Paratek Pharmaceuticals has a commitment to ensure that data are available to regulators and researchers when permitted, feasible and appropriate. Requests for data may be submitted to medinfo@paratekpharma.com.

Results

S. aureus cultures treated with 1/2 or 1/4 the MIC of omadacycline for 4 h showed haemolysis units/ 10^8 cfu of 47% and 59% of vehicle-treated cultures, respectively (Figure 1a and b). These values were comparable to those seen with 1/2 or 1/4 the MIC of comparator protein synthesis inhibitors: tetracycline (43% and 62%), clindamycin (41% and 40%) and linezolid (72% and 63%); MICs for respective antibiotics are listed in Table 1. In contrast, when cultures were treated with sub-growth inhibitory concentrations of cell wall inhibitor antibiotics, there was less inhibition of haemolysis with vancomycin (92% and 86% of vehicle-treated haemolysis units/cfu, with 1/2 MIC and 1/4 MIC, respectively), and there was even an increase in haemolysis with cephalothin (311% and 199% of vehicle-treated haemolysis units/cfu, with 1/2 MIC and 1/4 MIC, respectively). In washout experiments, exposure to as little as 1/4 of the MIC of omadacycline for 1 h decreased the haemolysis units/ 10^8 cfu by 60% for 4 h following removal of the drug (Figure 1c).

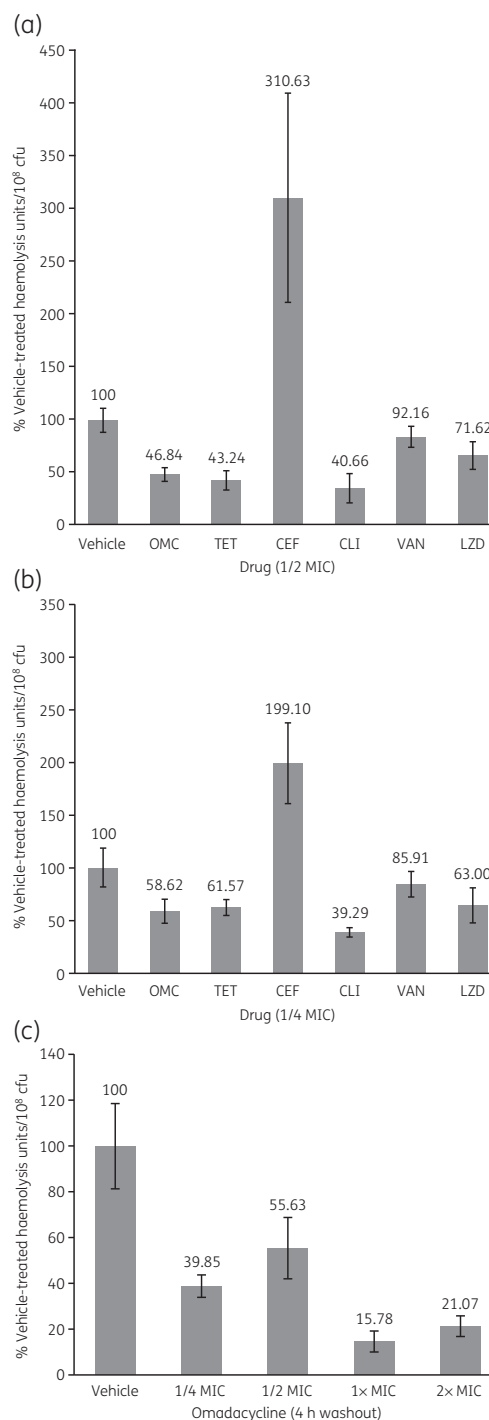


Figure 1. Haemolytic activity of *S. aureus* Wood 46 after 4 h growth with 1/2 MIC (a) and 1/4 MIC (b) of omadacycline (OMC), tetracycline (TET), cephalothin (CEF), clindamycin (CLI), vancomycin (VAN), or linezolid (LZD). Vehicle = 0.0003% DMSO in Mueller Hinton II (MH) broth. Data represent the mean of five cultures; error bars indicate standard deviations. Panel (c) shows haemolytic activity of *S. aureus* Wood 46 grown for 1 h with the indicated concentration of omadacycline, followed by 4 h growth without drug. Vehicle = 0.0025% DMSO in MH broth. Data represent the mean of three cultures; error bars indicate standard deviations. Values <100% indicate more inhibition of haemolytic activity than the vehicle control.

Table 1. MICs (mg/L) of omadacycline and comparator agents evaluated against *S. aureus* Wood 46

Antibiotic	MIC	1/2 MIC	1/4 MIC	1/8 MIC
Omadacycline	0.25	0.12	0.06	0.03
Tetracycline	0.12	0.06	0.03	0.015
Cephalothin	0.25	0.12	0.06	0.03
Clindamycin	0.06	0.03	0.015	0.008
Vancomycin	1	0.5	0.25	0.12
Linezolid	1	0.5	0.25	0.12

Discussion

Omadacycline inhibited *S. aureus* haemolytic activity *in vitro* at sub-growth-inhibitory concentrations, and inhibition was maintained for at least 4 h after removal of extracellular drug. This is the first study to demonstrate these effects with omadacycline and, to our knowledge, comparable data on the inhibition of haemolytic activity in this strain of *S. aureus* are not available for other tetracyclines. The effect of sub-growth-inhibitory concentrations of antibiotics on *S. aureus* virulence factor expression has been found to vary between drugs and among virulence factors.¹³ Previously, an antitoxin effect for *S. aureus* has been largely associated with clindamycin and more recently with linezolid, with limited information available on tetracyclines.^{7,14–16} However, most currently available protein synthesis inhibitors are less desirable monotherapy options for *S. aureus* infections due to resistance in *S. aureus* to clindamycin and azithromycin,¹⁷ safety concerns with other agents, including cardiac issues with azithromycin,¹⁸ *Clostridioides difficile* colitis associated with clindamycin,¹⁹ and myelosuppression caused by linezolid.²⁰ In future studies, it would be of interest to explore the effects of newer anti-ribosomal agents, such as tedizolid, in terms of suppression of haemolytic activity. Additionally, because it is not known how the suppression of haemolytic activity observed with omadacycline compares with that seen with other tetracyclines, such as doxycycline and minocycline, investigating this in future studies would also be worthwhile. An important limitation of the current study is that it examined haemolytic activity in a single strain of *S. aureus*, which is known to be a high-level producer of alpha toxin.²¹ The clinical relevance of the findings for *S. aureus* infections could be further investigated using strains of MRSA such as ST22, ST59, ST239, USA300 MRSA, or USA100. Additionally, analyses for other virulence factors, including those in group A streptococci, are warranted given the burden of toxin production for these infection types.

Conclusions

The suppression of virulence factors, in addition to the *in vitro* potency of omadacycline, may contribute to the efficacy of omadacycline for community-acquired bacterial pneumonia and acute bacterial skin and skin structure infections caused by virulent strains of *S. aureus*. This finding could apply to other organisms and other virulence factors that

require new protein synthesis to establish and enable the progression of disease.

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A.W.S., S.T.K. and K.W. are employees of Paratek Pharmaceuticals, Inc.; L.G.R. was an employee of Paratek Pharmaceuticals, Inc. at time of study.

Author contributions

All authors contributed to the design, analysis, and interpretation of the study, and approved the final article for publication.

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