

Comparative efficacy of omadacycline (PTK796) in lethal *Streptococcus pneumoniae* and *Staphylococcus aureus* pneumonia models

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ABSTRACT

Objective

Omadacycline is a novel aminomethylcycline with excellent activity against pulmonary pathogens and overcomes tetracycline resistance. The objective of these studies was to evaluate omadacycline as a clinical candidate to treat Gram-positive pneumonias.

Methods

Neutropenic (cyclophosphamide treated) male CD-1 mice were infected intranasally with 50µl containing approximately 7.9×10^7 CFU of *Staphylococcus aureus* USA300 (SA) diluted in sterile PBS. Neutropenic or immunocompetent male CD-1 mice were also infected intranasally with 50µl containing approximately 6.4×10^6 of *Streptococcus pneumoniae* PBS1339 (SP) diluted in sterile PBS. Animals were treated with omadacycline, comparator, or saline at 2 hours post-infection (pi) with a single intravenous (IV) dose and survival determined out to 7 days pi.

Results

Omadacycline was more effective than tigecycline and vancomycin in preventing death in both the SA and SP neutropenic pneumonia models at all time points tested (Table 1). Omadacycline was over 3 fold more effective than tigecycline at the early end points tested for both SA and SP, and 1.5-2 fold more effective by 7 days pi. Vancomycin was ineffective at any of the doses tested and at either of the time points for both SA and SP infections. In addition, doxycycline was tested against SA and daptomycin, linezolid, ceftriaxone, and levofloxacin were tested against SP. All these comparators failed by 7 days pi even at the highest doses tested (>18 mg/kg or higher). Omadacycline was more effective than linezolid or levofloxacin and as effective as tigecycline and vancomycin in preventing death in a SP non-neutropenic pneumonia model at 96 hours pi. Omadacycline was over 2 fold more effective in preventing death than vancomycin at day 7 pi.

Conclusion

Omadacycline was successful in protecting neutropenic or non-neutropenic (SP only) mice against a lethal SA or SP pneumonia and was equal or more effective than tigecycline or vancomycin. This data suggests that omadacycline may be considered as a potential candidate for the treatment of Gram-positive pneumonias, particularly in patients with normal immune function.

INTRODUCTION

Omadacycline (PTK796; OMC) is an aminomethylcycline, the first antibiotic in a new class of tetracycline derivatives, that exhibit excellent activity against human pathogens, including those resistant to commonly used antibiotics. Its spectrum of activity include Gram-positive and Gram-negative aerobes and anaerobes, and atypical pathogens. Omadacycline is being developed for both parenteral and oral clinical use in complicated skin and skin structure infections (cSSSI) and community-acquired bacterial pneumonia. Omadacycline has been shown to be well tolerated in Phase 2 clinical trials in cSSSI.

We compared omadacycline to clinically relevant comparators in neutropenic and non-neutropenic murine respiratory tract infection models to evaluate its potential as a therapy for *S. pneumoniae* and *S. aureus*-induced community-acquired pneumonia and to determine the effect of the immune status on the outcome of treatment. It is well known that most antibiotics benefit from the host response to infection. Evaluations in neutropenic compared to normal animal infection models help guide the development of antibiotics and further define the pharmacodynamic interactions between drug, bacteria, and host.

METHODS

Bacteria

Tetracycline-sensitive *Streptococcus pneumoniae* PBS1339 (GSK1629) and methicillin and tetracycline-resistant *Staphylococcus aureus* USA300 (ATCCBAA 1556) grown from frozen stocks was used for pulmonary infection models.

Animals

Six-week-old male CD-1 mice, weighing 18-30g (Charles River, Hartford, CT) were used for all experiments. Animals were allowed food and water *ad libitum* and kept at a constant 12 hour light/dark cycle. At any time, if any animals exhibited signs of severe illness, they were painlessly euthanized by isoflurane or CO₂ narcoses followed by cervical dislocation. All protocols were approved by the Paratek Pharmaceuticals, Inc. IACUC.

Antibiotics

Omadacycline (PTK796; OMC) was synthesized at Paratek Pharmaceuticals, Inc. Tigecycline was obtained from Bosche Scientific, New Brunswick, NJ. Linezolid and levofloxacin was purchased from Sequoia Research, Pangbourne, UK. Vancomycin HCL and ceftriaxone was purchased from Sigma-Aldrich Corp., St. Louis, MO. Daptomycin was acquired from Cubist Pharmaceuticals, Inc., Lexington, MA. Doxycycline was obtained from Hovione, East Windsor, NJ.

Acute Respiratory Tract Infection (RTI) Models

Mice were rendered neutropenic (if necessary) by injecting cyclophosphamide intraperitoneally at 150 and 100 mg/kg doses on days -4 and -1 pi, respectively. Following a 37°C overnight incubation, the colonies (*S. pneumoniae* on blood agar plates, CO₂ enriched environment) or broth (*S. aureus* in Mueller Hinton broth, 180 rpm) were aseptically collected and resuspended in sterile PBS. Mice were infected (n=5 mice/group) intranasally with 50µl of the bacterial suspension (final inoculum was $\sim 6.4 \times 10^6$ CFU/mouse for *S. pneumoniae* or $\sim 7.9 \times 10^7$ CFU for *S. aureus*). At 2 hours pi, mice were treated IV with a dose range of omadacycline or comparator in a vehicle of sterile saline for injection (see Figures for exact doses used). Each experiment also had an untreated control group. Mice were observed daily for 7 days pi. The PD₅₀ (protective dose, 50%), defined as the dose required to achieve 50% survival at 48 or 96 hours, and 7 days pi, was calculated using a nonlinear regression analysis using Prism 3.0 software. An earlier PD₅₀ could not be calculated for *S. pneumoniae* because 100% of the untreated control mice did not always succumb to infection before 96 hours pi. The *S. aureus* USA300 non-neutropenic model was attempted, but this strain was not virulent enough in control animals.

RESULTS

A single dose of omadacycline was effective in preventing death in the SP neutropenic pneumonia models by 96 hours and 7 days pi (Table 1). Omadacycline was over 3 fold more effective than all the comparators except ceftriaxone at the early end point (Figure 1) and nearly 2 fold more effective or more by 7 days pi for all the comparators. Vancomycin, linezolid, levofloxacin and daptomycin were ineffective at any of the doses tested and at either 96 hours or 7 days pi.

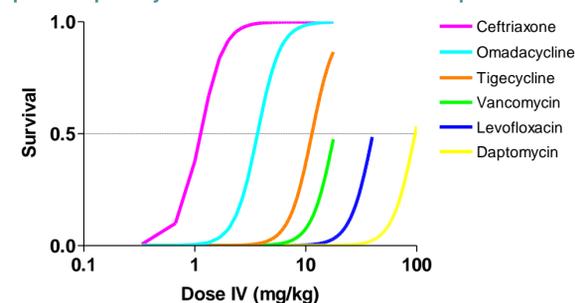
Table 1. Efficacy of omadacycline versus clinical comparators in a neutropenic lethal *S. pneumoniae* respiratory tract infection model

Drug	MIC (mg/L)	<i>S. pneumoniae</i> PBS1339	
		PD ₅₀ (mg/kg)(95% CI)	
		96 hr pi	7 day pi
Omadacycline	0.125	3.53 ± 1.46 SD*	10.20 ± 3.05 SD*
Tigecycline	0.125	11.38 (9.38, 13.39)	19.92 (19.14, 20.70)
Vancomycin	0.25	>18	>18
Daptomycin	0.25	>100	>100
Linezolid	1	>18	>18
Ceftriaxone	0.015	1.13 (0.56, 1.70)	>18
Levofloxacin	0.25	>40	>40

CI=Confidence Interval; SD=Standard Deviation, * Mean of 5 individual experiments. Values without confidence intervals had PD₅₀ values that exceeded the highest dose tested and were not able to be estimated by the Prism 3.0 software

RESULTS (continued)

Figure 1. Nonlinear regression survival curves for the *S. pneumoniae* neutropenic respiratory tract infection model at 96 hours pi



All compounds, except for levofloxacin and the daptomycin experiment, were tested at 0.33, 1, 3, 9, and 18 mg/kg. Levofloxacin was tested at 1, 5, 10, 20 and 40 mg/kg. In the daptomycin experiment, daptomycin was tested at 6.25, 12.5, 25, 50 and 100 mg/kg and omadacycline at 0.78, 1.56, 3.13, 6.25, and 12.5 mg/kg to accommodate the lower efficacy of daptomycin in lung infections.

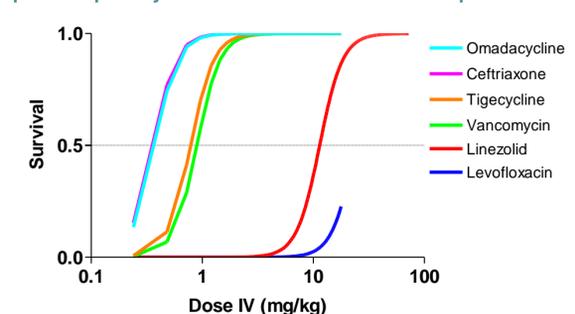
The efficacious dose required was significantly lower in the non-neutropenic pneumonia model than in the neutropenic model for all the drugs studied. Less than 1/6 of the omadacycline dose was required in the non-neutropenic model to achieve efficacy at the 96 hour and 7 day endpoints (Table 2). Even so, a single dose of omadacycline was over 10 fold more effective than linezolid and levofloxacin (Figure 2). Omadacycline was equally as effective as tigecycline, vancomycin and ceftriaxone at 96 hour pi. Omadacycline was equally as effective as tigecycline, slightly more potent than vancomycin, and slightly less potent than ceftriaxone at 7 days pi.

Table 2. Efficacy of omadacycline versus clinical comparators in a non-neutropenic lethal *Streptococcus pneumoniae* pneumonia model

Drug	MIC (mg/L)	<i>S. pneumoniae</i> PBS1339	
		PD ₅₀ (mg/kg)(95% CI)	
		96 hr pi	7 day pi
Omadacycline	0.125	0.58 ± 0.71 SD*	1.24 ± 0.48 SD*
Tigecycline	0.125	0.69 ± 0.27 SD*	0.91 ± 0.00 SD*
Vancomycin	0.25	0.90 (0.37, 1.42)	3.30 (3.23, 3.35)
Linezolid	1	11.42 (6.99, 15.84)	38.81 (23.14, 54.48)
Ceftriaxone	0.015	0.36 (0.11, 0.62)	0.37 (0.25, 0.48)
Levofloxacin	0.25	>18	>18

CI=Confidence Interval; SD=Standard Deviation, * Mean of 2 individual experiments. Levofloxacin survival was low even at the highest dose tested therefore Prism 3.0 could not estimate a PD₅₀ value.

Figure 2. Nonlinear regression survival curves for the *S. pneumoniae* non-neutropenic respiratory tract infection model at 96 hours pi



All compounds, except for linezolid, were tested at 0.11, 0.33, 1, 3, 9, and 18 mg/kg. Linezolid was tested at 1, 3, 9, 18, 36 and 72 mg/kg.

RESULTS (continued)

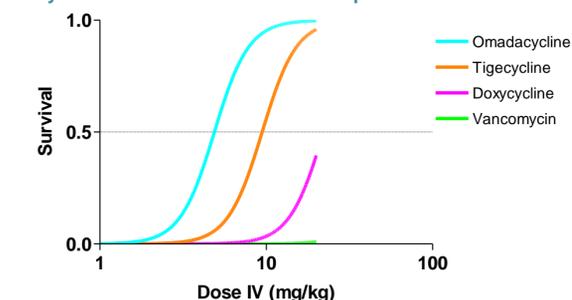
In the MRSA neutropenic respiratory tract infection model, omadacycline was slightly more effective than tigecycline at 48 hours and 7 days pi. Both doxycycline and vancomycin were not efficacious at either endpoint even at the highest dose tested (20 mg/kg or higher).

Table 3. Efficacy of omadacycline versus clinical comparators in a neutropenic lethal *Staphylococcus aureus* pneumonia model

Drug	MIC (mg/L)	<i>S. aureus</i> USA300	
		PD ₅₀ (mg/kg)(95% CI)	
		48 hr pi	7 day pi
Omadacycline	0.25	2.81 (2.05, 3.57)	4.88 (2.82, 6.94)
Tigecycline	0.125	9.45 (7.91, 11.00)	9.45 (7.91, 11.00)
Doxycycline	1	>20	>20
Vancomycin	0.5	>40	>40

CI=Confidence Interval; SD=Standard Deviation. Values without confidence intervals had PD₅₀ values that exceeded the highest dose tested and were not able to be estimated by the Prism 3.0 software

Figure 3. Nonlinear regression survival curves for the *S. aureus* neutropenic respiratory tract infection model at 48 hours pi



All compounds were tested (n=5/group) at 0.625, 1.25, 2.5, 5, 10, and 20 mg/kg.

CONCLUSIONS

- In the neutropenic *S. pneumoniae* pulmonary infection model, omadacycline, tigecycline and ceftriaxone performed well although the long-term efficacy for all drugs was reduced.
- Omadacycline and the comparator agents all benefited substantially from the presence of an intact immune system. All exhibited substantially reduced dose requirements for efficacy and benefit was observed for long-term survival.
- Linezolid and levofloxacin were much less effective vs. *S. pneumoniae* than omadacycline, tigecycline, ceftriaxone, and vancomycin indicating that the pharmacodynamic requirements for efficacy were not achieved by a single intravenous dose in either neutropenic or normal mice. Vancomycin was ineffective in the neutropenic pneumonia model but benefited substantially from an intact immune system.
- Omadacycline and tigecycline were superior to doxycycline and vancomycin in the *S. aureus* pulmonary model in neutropenic mice. The inability of the *S. aureus* isolate to cause a lethal pulmonary infection in non-neutropenic animals, prevented the evaluation of the contribution of the immune system in the treatment of this infection.
- These results support the development of omadacycline in bacterial pneumonia caused by *S. pneumoniae* and *S. aureus*, particularly in patients with normal immune function. The role of the immune system on the response to therapy should be carefully considered in the determination of the pharmacodynamic relationship of drug, pathogen, and host.