

Efficacy of PTK796 in a Rat MRSA Infective Endocarditis (IE) Model

D. Yu, K.A. Kelley, M.N. Osborn, A. Trzasko, S. Pettiford, D. McKenney, P.A. Bradford — Department of Infectious Diseases, Novartis Institutes for BioMedical Research, Inc., Cambridge, MA, USA

ABSTRACT

Background

PTK796 is the first semi-synthetic aminomethylcycline in clinical development. It is active *in vitro* and *in vivo* against resistant pathogens, particularly MRSA and VRE. The efficacy of PTK796, tigecycline (TGC) and daptomycin (DAP) were tested using a rat infective endocarditis (IE) model with a MRSA 32 strain.

Methods

IE was induced following transcarotid-transaortic valve indwelling catheterization after i.v. infection with 5×10^5 CFU/rat MRSA 32 strain. At 6 hours and the next 4 days post-infection, animals were randomized to receive: i) saline s.c. qd; ii) PTK796 s.c. bid; iii) TGC s.c. bid; or i.v.) DAP s.c. qd. At 18 h after the last treatment, hearts and spleens were removed and quantitatively cultured. The ends of catheters from the left ventricle were qualitatively cultured.

Results

All dosing regimens significantly decreased bacterial counts in all target tissues ($P < 0.05$). ED₅₀ (the dose at which 50% of the animals showed a 3-log reduction in bacterial load relative to the colony counts in saline-treated controls) of PTK796 (bid), TGC (bid), and DAP (qd) were 2.89, 0.97, and below 2.5 mg/kg, respectively.

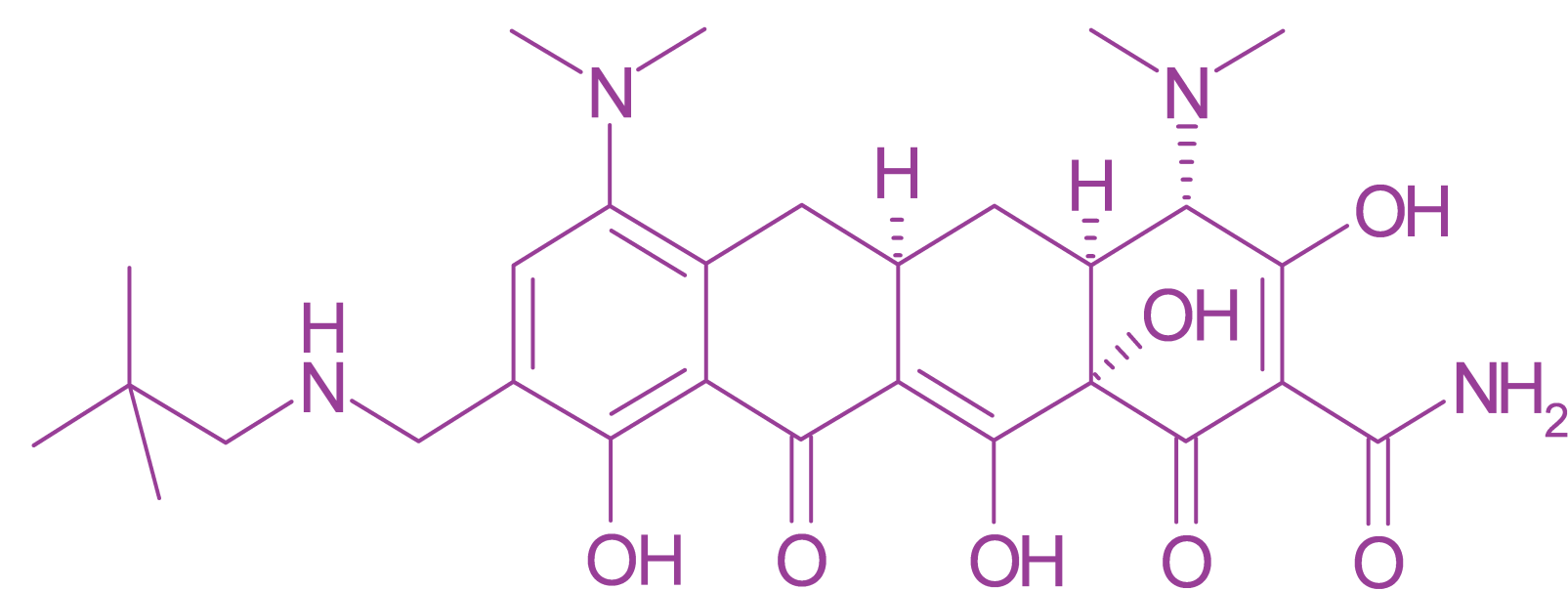
Conclusions

PTK796 showed efficacy against MRSA 32 strain-induced IE in rats.

INTRODUCTION

PTK796 is the first semi-synthetic aminomethylcycline in clinical development for both i.v. and oral indications. PTK796 is active *in vitro* and is effective *in vivo* against pathogens resistant to tetracycline and other antibiotics, including methicillin, vancomycin, erythromycin, and ciprofloxacin. Indeed, PTK796 has potential clinical significant activity against Gram-positive and Gram-negative bacteria, as well as anaerobic and atypical pathogens such as *Legionella* species. Thus, PTK796 holds promise as an option in the treatment of the serious acute and multiresistant bacterial pathogens that cause infections that are becoming an increasing problem in the clinic.

Figure 1. Chemical structure of PTK796.



METHODS

MIC Testing

- The minimum inhibitory concentration (MIC) of PTK796 and other antibiotics against the strain used in these studies was determined by microbroth dilution in fresh media according to standard Clinical and Laboratory Standards Institute (CLSI) protocols

Animals

- Male Sprague-Dawley rats weighing 230–250 g were intracardioventricular cannulated 5 days prior to infection

Rat Endocarditis Infection

- Methicillin-resistant *Staphylococcus aureus* (MRSA) strain 32 was inoculated in Mueller Hinton broth and incubated at 37°C with 150 rpm shaking overnight. Overnight cultures were further diluted in normal saline to obtain the target concentration. Rats were infected with 0.5 mL inoculum containing approximately 5×10^5 colony-forming units (CFU) through tail vein injection

Antibiotics Tested and Dosing Regimens

- PTK796 was synthesized at Paratek Pharmaceuticals (Boston, MA). Daptomycin (DAP) was purchased from Cubist Pharmaceuticals (Lexington, MA). Tigecycline (TGC) was purchased from Wyeth Pharmaceuticals. All rats were first treated at 6 hours post-infection with saline (2 mL/kg s.c.), PTK796 (5, 1.67, or 0.56 mg/kg s.c.), tigecycline (5, 1.67, or 0.56 mg/kg s.c.), or daptomycin (10, 5, or 2.5 mg/kg s.c.); PTK796 and tigecycline were then administered twice daily (bid) and saline and daptomycin were administered once daily (qd) over the next 4 days

Determination of Bacterial Levels

- All rats that survived the study period were terminated on day 5. Hearts and spleens were removed, weighed, and homogenized, then plated for bacterial enumeration. Catheter placements were confirmed at the time of harvest and rats that did not have a suitable placement of the catheter were not included in the analysis of the data. A small portion of catheters from the left ventricle were cut and tissue was plated on blood agar plates for qualitative culture

Analysis of Results

- Bacterial levels in heart were enumerated and an effective dose 50 (ED₅₀) value where 50% of the animals treated with PTK796 or comparators showed a 3-log reduction in bacterial load compared with vehicle-treated animals was calculated by Probit analysis using Graphpad Prism 5 software

RESULTS

Table 1. MIC of PTK796, tigecycline (TGC), and daptomycin (DAP) against methicillin-resistant *Staphylococcus aureus* strain 32 (MRSA 32).

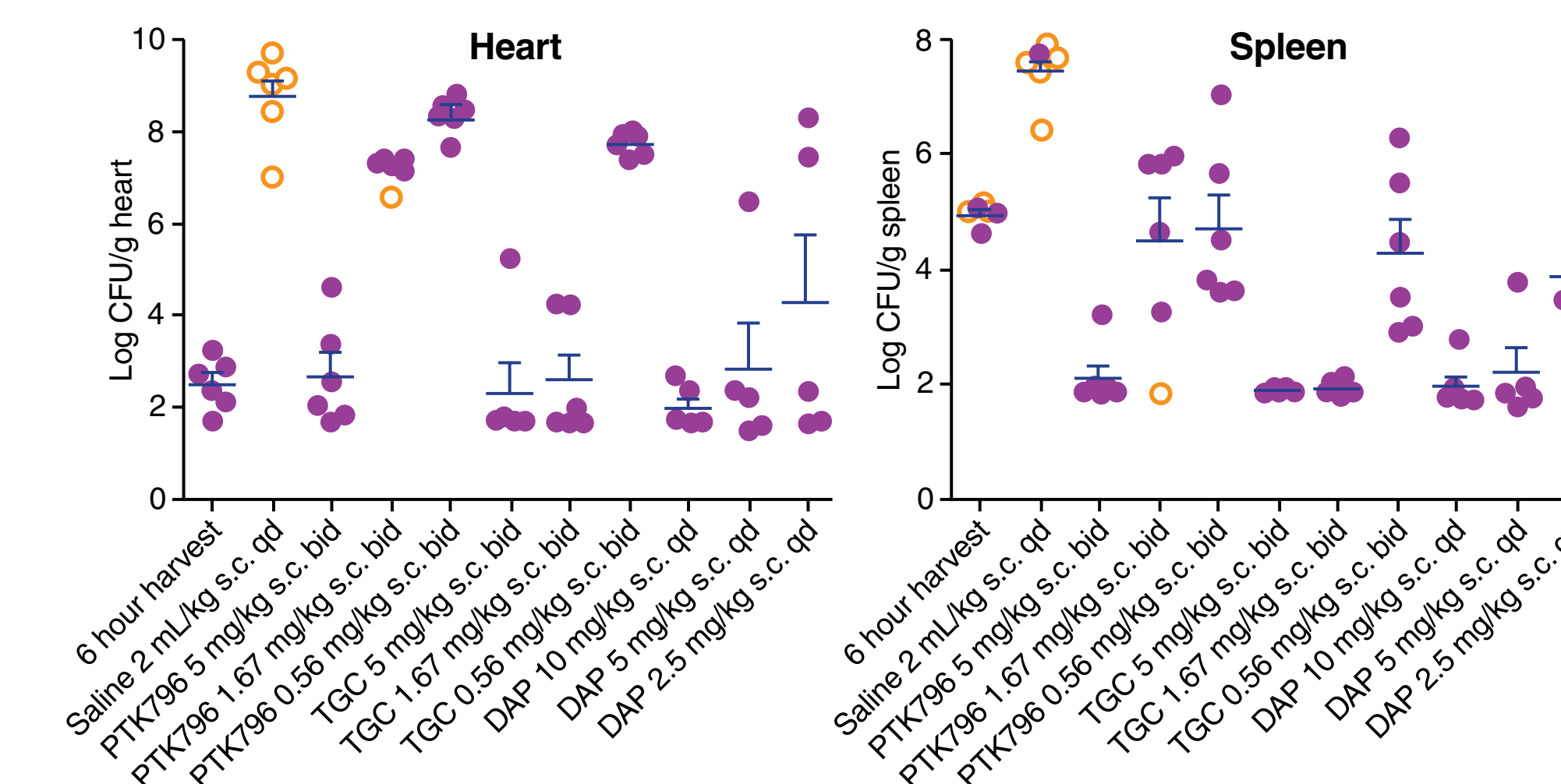
MIC (μg/ml)	PTK796	TGC	DAP
MRSA 32	0.5	0.125	0.125

Table 2. Efficacy of PTK796 and its comparators against MRSA-induced rat IE model.

Group	Catheter culture positive at harvest	5 days survival	No. of sterile hearts	Log CFU/g heart		Log CFU/g spleen		Heart 3-log reduction ED ₅₀ (mg/kg/dose) (95% CI)
				Mean	SD	Mean	SD	
6 hour p.i. control	2/6	NA	1/6	2.46	0.56	4.97	0.17	
Saline 2 mL/kg s.c. qd	6/6	0/6	0/6	8.76	0.97	7.48	0.52	
PTK796 5 mg/kg s.c. bid	4/6	6/6	2/6	2.64	1.14	2.09	0.54	2.89 (0.59, 5.19)
PTK796 1.67 mg/kg s.c. bid	6/6	5/6	0/6	7.16	0.32	4.54	1.71	
PTK796 0.56 mg/kg s.c. bid	6/6	6/6	0/6	8.34	0.40	4.71	1.39	
TGC 5 mg/kg s.c. bid	1/6	6/6	5/6	2.27	1.44	1.87	0.03	0.97 (0.20, 1.04)
TGC 1.67 mg/kg s.c. bid	1/6	6/6	3/6	2.53	1.30	1.92	0.12	
TGC 0.56 mg/kg s.c. bid	6/6	6/6	0/6	7.71	0.23	4.28	1.40	
DAP 10 mg/kg s.c. qd	1/6	6/6	4/6	1.93	0.44	1.94	0.41	
DAP 5 mg/kg s.c. qd	1/5	5/5	2/5	2.78	2.08	2.18	0.91	<2.5
DAP 2.5 mg/kg s.c. qd	3/5	5/5	2/5	4.25	3.32	3.88	1.61	

bid, twice daily; CFU, colony-forming unit; CI, confidence interval; DAP, daptomycin; ED₅₀, effective dose 50; IE, infective endocarditis; MRSA, methicillin-resistant *Staphylococcus aureus*; p.i., post-infection; s.c., subcutaneous; SD, standard deviation; TGC, tigecycline; qd, once daily.

Figure 2. Efficacy of PTK796 and its comparators against MRSA-induced rat IE model (open circles = animal euthanized or found dead before the end of the study). IE, infective endocarditis; MRSA, methicillin-resistant *Staphylococcus aureus*.



CONCLUSIONS

- The rat infective endocarditis (IE) model provides a good system for evaluating antibiotics used for serious infections
- PTK796 showed good efficacy against a strain of MRSA (susceptible to all the antibiotics tested) in the IE model, reducing bacterial counts in the heart by over 5 log₁₀ CFU/g at a dose of 5 mg/kg
- PTK796 was less efficacious than tigecycline in the model, especially in the ratio of total heart sterilization (2/6 for PTK796 at 5 mg/kg vs. 5/6 for the same dose of tigecycline)
- The efficacy of daptomycin in the model was less than the lowest dose tested; therefore a direct comparison with PTK796 cannot be made
- The efficacy of PTK796 in this MRSA-induced rat IE model indicates the potential utility of PTK796 in Gram-positive infections

DISCUSSION

- Doses selected for this study were based on historical animal study data, not related to human doses
- The particular MRSA strain we used in this study does not generate obvious vegetation at the valve. We hypothesized that using whole heart counts instead of valve counts would make the data more reliable
- Once the endocarditis was established, bacteria counts in heart were usually at least 1-2 log higher than in spleen, unless sterile. We monitored spleen bacterial counts only to show the bacteremia level. Unlike in the sepsis model, endocarditis animals do not die shortly after infection unless due to heart attack