Poster 180
F1-2161

50th Annual ICAAC
12-15 September, 2010
Boston, MA

TP-434 is Highly Efficacious in Animal Models of Infection

T. MURPHY, A. SLEE, J. SUTCLIFFE[2]

[1]ViviSource, Waltham, MA and [2]Tetraphase Pharmaceuticals Inc., Watertown, MA

Contact:
Leland Webster
Tetraphase Pharmaceuticals
lwebster@tphase.com

Abstract

Background: TP-434 is a novel broad-spectrum fluorocycline being developed for a wide range of infections.
Methods: CD-1 female mice were infected IP for septicemia models; compound was dosed 1 hr post-challenge, and survival was recorded 48 hrs post-challenge. Mice were rendered neutropenic before challenge in the thigh; treatment was IV 1.5 hr post-challenge, with bacterial burden reduction recorded 24 hr post-challenge. For lung models, mice were made neutropenic and infected intranasally with either MRSA or *Streptococcus pneumoniae* and dosed IV 2 and 12 hrs post-challenge, with lung bacterial burden determined 24 hrs post-infection. For UTI models, mice were infected IV with a preoptimized bacterial load and compound was administered IV at 12 and 24 hours post-infection. At 36 hours post-infection, kidneys were collected and cultured for colony-forming units.
Results: TP-434 was highly effective in septicemia models, demonstrating PD_{50}s of ≤1 mg/kg against *S. aureus*, including tetracycline-resistant strains, MRSA and *S. pyogenes*. The PD_{50}s against *E. coli* were 1.2-4.4 mg/kg. In lung models, TP-434 IV 10 mg/kg reduced MRSA in the lung equivalent to linezolid PO 30 mg/kg, and at IV doses of 3-12 mg/kg TP-434 was more efficacious than linezolid PO 30 mg/kg vs *S. pneumoniae*. TP-434 was highly efficacious at 5 mg/kg IV and 20 mg/kg IV, providing a 4- and 2.5-log drop in kidney bacterial burden in models challenged with uropathogenic *E. coli* or an extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*, respectively. Neutropenic thigh model results are shown in the poster.
Conclusion: TP-434 is highly efficacious in multiple murine models of infection against clinically important multidrug-resistant gram-positive and gram-negative pathogens.

Introduction

TP-434 is a novel fully synthetic tetracycline-class antibiotic that was designed to have a broad antibacterial spectrum with potent activity against problematic multidrug-resistant gram-negative and gram-positive bacteria (see posters F1-2155, 2157, 2158, 2160, and 2163). TP-434 will be used for treatment of serious hospital infections as it is unaffected by resistance mechanisms to other antibiotics and is specifically active against tetracycline-resistant strains (see posters F1-2158 and F1-2160). The pharmacokinetics in animal species (poster F1-2163) and man (A1-027 and A1-028) have been determined. The preclinical and clinical data support advancement of TP-434 into Phase 2 studies.

Materials & Methods

Antibiotics. TP-434 and tigecycline were provided by Tetraphase Pharmaceuticals and dosed intravenously. Linezolid oral suspension (dosed PO) and vancomycin (dosed IV) were provided by Henry Schein Veterinarian Supply (Melville, NY) and Sigma Aldrich (St. Louis, MO) respectively.
Media components. TSA, TSA-II agar plates, Brain Heart Infusion Broth (BHI) were from BBL (Franklin Lakes, NJ). Cyclophosphamide, carrageenen, and type III hog gastric mucin were obtained from Sigma Aldrich.
***In vitro* MICs**. Minimum inhibitory concentrations for isolates were performed according to CLSI standards using the broth micro-dilution method.
Animals. All studies were performed under approved IACUC protocols and conform to OLAW standards. Female mice, CD-1 or Balb/C, purchased from Charles River Laboratories, Inc. (Wilmington, MA) were acclimated for 5 days prior to start of studies. Animals had free access to food and water throughout the study as well as provided enrichment.
Bacteria preparation for *in vivo* infections. Bacteria were prepared from an overnight plate culture by re-suspending in saline and adjusting the suspension to a 0.1 OD at 625 nm of a 1:10 dilution. For systemic infection studies, bacteria were diluted either in BHI broth or hog gastric mucin for injection. For lung, thigh and urinary tract infection studies, bacterial inocula were prepared in saline for administration. Plate counts were performed to determine actual colony-forming units (CFU).
Mouse Systemic Infection Studies. Mice received treatment *via* intravenous injection 1 hour post IP infection. At termination of study, percent survival was calculated and the dose (mg/kg) effecting 50% survival, the protective dose 50% (PD_{50}), was reported along with 95% confidence intervals as calculated by Probit analysis using GraphPad Prism version 4.03 (GraphPad Software).
Neutropenia induction. For the lung and thigh infection studies, mice were rendered neutropenic through two consecutive IP injections of cyclophosphamide of 150 and 100 mg/kg on days -4 and -1, respectively.
Mouse Thigh Infection. Mice were infected with approximately $5x10^5$ CFU/ml of bacteria in a 0.1 ml volume into the right thigh. At 1.5 hours post infection mice received treatment *via* intravenous injection. One group of infected mice were euthanized and thigh processed for bacterial titers to serve as T=0 controls. Twenty-four hours post treatment, the remaining mice were euthanized, thighs aseptically removed, weighed, homogenized, serially diluted and plated on bacterial growth media. CFUs per gram of thigh were calculated after overnight incubation of bacterial plates. The amount of test article required to achieve 1, 2, and 3 log_{10} reductions from 24 hour control thighs was calculated.
Mouse Lung Infection. Mice, under light anesthesia, were inoculated with 50 µL of the prepared bacterial inoculum *via* intranasal inhalation. Mice received treatment *via* tail vein IV or *via* oral gavage (linezolid) at 2 and 12 hours post infection. Twenty-four hours post treatment, mice were euthanized, lungs aseptically removed, homogenized, serially diluted and plated on bacterial growth agar. After overnight incubation, colonies were counted and CFU/ gram of lung were determined.
Kidney Burden Infection Model. Mice were infected with 0.2 mL of prepared bacterial inoculum *via* intravenous injection. Infections utilizing *Klebsiella pneumoniae* KP453 were prepared with 0.2% Carrageenan for injection. At 12 and 24 hours post infection animals received treatment *via* IV. 18 hours post treatment, mice were euthanized, kidneys aseptically removed, weighed, homogenized, serially diluted and plated on bacterial growth media. CFU per gram of kidney was calculated after overnight incubation. The change in CFUs from infection controls was calculated.

Results

Table 1. The efficacy of TP-434 and comparators in the Neutropenic Thigh Model

Test Article	Strain	MIC µg/ml	Dose (mg/kg) Providing 2 log_{10} Reduction
TP-434	*S. aureus* Smith ATCC 13709	0.06	0.2
Tigecycline		0.06	1.5
TP-434	MRSA SA161 *tet*(M)	0.125	1
Tigecycline		0.125	12.5
Vancomycin		1	2.9
TP-434	*S. aureus* SA158 *tet*(K)	0.125	8.2
Tigecycline		0.06	5.4
Vancomycin		2	>20
TP-434	MRSA SA192 USA300 *tet*(K)	0.25	9.5
Tigecycline		0.125	8
Vancomycin		1	19.2
TP-434	*S. pyogenes* ATCC 8668	0.016	9
Tigecycline		0.016	15.8
Linezolid		2	>20

Table 2. The efficacy of TP-434 and comparators in Septicemia Model with Intraperitoneal Challenge

	IV PD_{50} in mg/kg (95% CI)						
Test Article	*S. aureus* ATCC 13709	MRSA SA161 *tet*(M)	MRSA USA300 *tet*(K)	*S. pyogenes* ATCC 8668	*S. pyogenes* ATCC 19615	*E. coli* ATCC 25922	*E. coli* EC133 ESBL+(SHV) *tet*(B)
TP-434	0.3 (0.29-0.31)	1.0 (0.56-1.4)	0.3 (0.13-0.47)	1.0 (0.78-1.2)	~0.05 (---)	4.36 (0.01-8.72)	1.2 (0.84-1.6)
Tigecycline	0.32 +/- 0.07[a]	1.0 (0.78-1.2)	0.35 (0.34-0.37)	2.5 (1.7-3.4)	0.3 (0.04-0.56)	1.74 (0.91-2.57)	3.5 (2.4-4.7)
Tetracycline	NT	>10	>10	NT	NT	NT	NT
Vancomycin	NT	NT	0.3 (0.15-0.45)	NT	NT	NT	NT
Linezolid	NT	NT	NT	>10	0.63 (0.06-1.2)	NT	NT
Imipenem	NT	NT	NT	NT	NT	NT	<0.3

[a]Mean and standard deviation of PD_{50} from three experiments; NT = not tested; CI = confidence interval; ESBL+ = extended-spectrum β-lactamase producing; SHV = type of ESBL

Table 3. MICs of TP-434 and Comparators for Strains used in Murine Mouse Models

	MIC in µg/ml										
Test Article	*S. aureus* ATCC 13709	MRSA SA161 *tet*(M)	MRSA SA192 *tet*(K) USA300	MRSA SA191 *tet*(M)	*S. pyogenes* ATCC 8668	*S. pyogenes* ATCC 19615	*E. coli* ATCC 25922	*E. coli* EC133 ESBL+ *tet*(B)	*E. coli* EC200 *tet*(B)	*S. pneumoniae* SP160	*K. pneumoniae* KP453
TP-434	0.063	0.063	0.25	0.5	0.016	0.016	0.25	0.13	0.13	≤0.016	0.5
Tigecycline	0.063	0.13	0.13	0.25	0.016	0.016	0.13	0.13	0.13	0.03	0.5
Tetracycline	0.25	>32	64	>64	0.25	0.063	1	>32	>64	32	8
Vancomycin	1	1	1	1	0.5	0.5	NT	NT	NIA	0.25	NIA
Linezolid	4	2	1	2	2	1	NT	NT	NIA	0.5	NIA
Imipenem	NT	NT	NIA	NT	NT	NT	0.25	0.13	0.25	NT	0.5/0.03[a]

[a] MIC to meropenem ; NIA = not intrinsically active; NT = not tested

Figure 1. The Efficacy of TP-434 and Comparators in Murine Lung Models

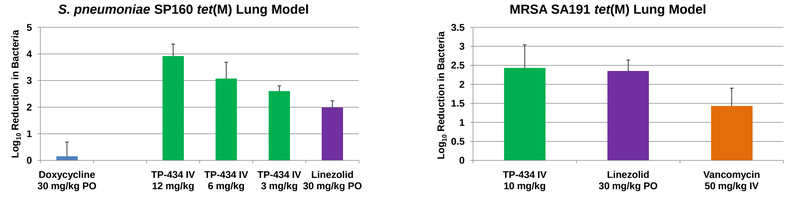
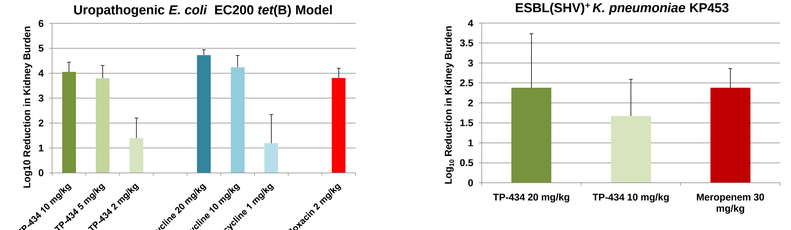


Figure 2. The Efficacy of TP-434 and Comparators in Murine UTI/Pyelonephritis Models



Conclusions

- **TP-434 was highly efficacious against multidrug-resistant *E. coli* and *K. pneumoniae* in septicemia with peritoneal challenge and UTI/pyelonephritis models**
- **TP-434 provided significant protection in neutropenic thigh, septicemia with peritoneal challenge, and pneumonia models when challenge bacteria were MRSA, *S. pneumoniae*, or *S. pyogenes***
- **TP-434 was protective against both tetracycline-specific efflux [*tet*(K), *tet*(B)] and ribosomal protection mechanisms [*tet*(M)] and not impacted by resistance to other antibiotic classes**
- ***in vivo* efficacy from this poster, along with *in vitro* potency (F1-2158) and clinical data (A1-027, A1-028) support advancement of TP-434 into Phase 2 studies**