Poster
2445
114th ASM General Meeting
17-20 May 2014
Boston, MA

TP-271 is a Novel Fluorocycline Active Against Susceptible and Multidrug-Resistant *Neisseria gonorrhoeae*

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Revised Abstract

Background: Gonococcal disease caused by *Neisseria gonorrhoeae* is a common cause of urethritis in men and endocervicitis in women. Drug resistance is a serious concern, with alarming resistance trends diminishing the effectiveness of all current standard of care antibiotics, including fluoroquinolones, current tetracyclines, cephalosporins, aminoglycosides and macrolides. TP-271, a novel and fully synthetic tetracycline analog, is a potent broad-spectrum antibiotic in preclinical development for the treatment of community-acquired respiratory infections and use against infections caused by bacterial biothreats. To explore the spectrum of activity, TP-271 was tested *in vitro* against a panel of 20 *N. gonorrhoeae* isolates with various drug-resistance profiles. **Method:** Susceptibility testing was conducted by agar dilution according to CLSI guidelines; control antibiotics were tetracycline, penicillin, ciprofloxacin and ceftriaxone. Strains were screened by PCR for tetracycline-resistance determinants commonly found in *N. gonorrhoeae*. **Results:** The minimal inhibitory concentration (MIC) range for TP-271 was 0.03 – 0.5 µg/mL and $MIC_{50/90}$ was 0.13/0.5 µg/mL. The $MIC_{50/90}$ values for tetracycline, penicillin, ciprofloxacin and ceftriaxone were 1/8, 0.5/2, 0.008/>2, and ≤0.008/0.016 µg/mL, respectively. The activity of TP-271 was similar in strains displaying single or multiple resistance phenotypes. TP-271 showed potent activity against all eight tetracycline-resistant isolates (MIC range: 0.06 – 0.5 µg/mL), of which four were confirmed to be *tet*(M)-positive. Fourteen isolates were also confirmed to have an *rpsJ* mutation encoding a V57M variation in ribosomal protein S10 shown to reduce tetracycline activity (Hu, et al. 2005. AAC 49:4327). **Conclusions:** If confirmed *in vivo,* these data support TP-271 as a promising new antibiotic for use against infections caused by multidrug-resistant *N. gonorrhoeae*.

Background

- Urethritis in men and endocervicitis in women are serious sexually transmitted diseases caused by *N. gonorrhoeae* infections in which the standard-of-care antibiotics, including tetracyclines, cephalosporins, aminoglycosides and macrolides, have become alarmingly less effective due to the rapid rise in drug resistance. The U.S. Centers for Disease Control and Prevention (CDC) has prioritized drug-resistant *N. gonorrhoeae* as an urgent threat and estimates that there are 246,000 cases of drug-resistant *N. gonorrhoeae* infections annually (1).
- TP-271 is a novel broad-spectrum fluorocycline antibiotic with excellent potency against serious and multidrug-resistant Gram-negative, Gram-positive pathogens, anaerobic, and atypical pathogens. TP-271 is active against strains expressing all major mechanisms of antibiotic resistance, including tetracycline-specific efflux, ribosomal protection and drug-inactivation mechanisms.
- Target-based tetracycline resistance has been identified in *N. gonorrhoeae* (2), and more recently in *Klebsiella pneumoniae* (3). Mutations in *rpsJ*, which encodes the ribosomal protein S10, are associated with increased resistance to tetracyclines.
- The purpose of this study is to evaluate the activity *in vitro* of TP-271 against *N. gonorrhoeae*, including penicillin-, ciprofloxacin- and tetracycline-resistant (*tet*(M) and *rpsJ* mutants) clinical isolates.

Methods

Strains and Growth Conditions. *Neisseria gonorrhoeae* strains were isolated from a variety of geographical areas, specimen sources and spanning time periods from the 1970s, 1990s, and more recently 2005/2006. Strains were acquired from the laboratory of Dr. Ann Jerse (Uniformed Services University, Bethesda, MD) and from Eurofins-Medinet (Chantilly, VA). *N. gonorrhoeae* isolates were grown on chocolate agar plates for inoculum preparation. GC medium base agar (Difco #228950; BD, Franklin Lakes, NJ) supplemented with 1% IsovitaleX (BD #211876; BD, Franklin Lakes, NJ) was used for susceptibility testing per CLSI guidelines (4,5); plates were incubated at 37º C in 5% CO_2. *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC29213 were included as controls and were grown on Tryptic Soy Agar for inoculum preparation; GC agar supplemented with IsovitaleX was used for susceptibility testing. Only data for *N. gonorrhoeae* is shown in Table 1.

Determination of Minimal Inhibitory Concentrations (MICs). MICs were determined using the CLSI agar dilution method (4,5), with replicate plating of the organisms onto a series of agar plates of increasing concentrations of compound from 0.004 µg/mL to 16 µg/mL for TP-271, penicillin and tetracycline, from 0.008 µg/mL to 16 µg/mL for ceftriaxone and from 0.001 µg/mL to 2 µg/mL for ciprofloxacin.

Detection of Tetracycline-Resistance Genes by Polymerase Chain Reaction (PCR). The presence of tetracycline resistance genes previously reported in *N. gonorrhoeae (*6), the more recently reported tetracycline resistance determinant *rpsJ* (2), as well as *tet* (A) was assayed by PCR according to the methods in Grossman *et al* (7) using sequences described in Table 5.

Results

Table 1. Susceptibility of *N. gonorrhoeae*

Strain	MIC (µg/mL) TP-271	TET	PEN	CIP	CRO	Phenotype	Relevent Genotype
NG648	0.03	0.13	0.13	0.008	≤0.008		
NG649	0.03	0.13	≤0.016	0.008	≤0.008		
NG650	0.03	0.13	0.03	0.008	≤0.008		
NG659	0.03	0.06	0.13	0.008	≤0.008		
NG660	0.03	1	1	0.008	0.016		*rpsJ* V57M
NG658	0.06	8	0.25	0.008	≤0.008	Tet-R	*tet* (M)
NG885	0.06	8	2	2	≤0.008	Tet-R, Pen-R, FQ-R	*tet* (M), *rpsJ* V57M
NG652	0.13	0.25	1	0.008	≤0.008		*rpsJ* V57M
NG654	0.13	0.5	2	0.008	≤0.008	Pen-R	*rpsJ* V57M
NG656	0.13	0.5	0.25	0.008	≤0.008		*rpsJ* V57M
NG886	0.13	16	>16	>2	≤0.008	Tet-R, Pen-R, FQ-R	*tet* (M), *rpsJ* V57M
NG887	0.13	4	0.25	0.004	0.03	Tet-R**	*tet* (M)
NG651	0.25	1	1	0.008	0.016		*rpsJ* V57M
NG653	0.25	1	1	0.008	0.016		*rpsJ* V57M
NG655	0.25	0.5	1	0.008	0.016		*rpsJ* V57M
NG657	0.25	0.5	8	0.008	≤0.008	Pen-R	*rpsJ* V57M
NG661	0.5	2	1	0.008	0.016	Tet-R**	*rpsJ* V57M
NG888	0.5	2	2	>2	0.03	Tet-R**, Pen-R, FQ-R	*rpsJ* V57M
NG889	0.5	2	1	>2	0.016	Tet-R**, FQ-R	*rpsJ* V57M
NG890	0.5	2	1	>2	0.016	Tet-R**, FQ-R	*rpsJ* V57M

Indicates at or above CLSI breakpoint for resistance
* Tet-R: tetracycline-resistance, Pen-R: penicillin-resistance, FQ-R: fluoroquinolone-resistance
** no PCR evidence was seen for Tet-R genes *tet(A)*, *tet*(B), *tet(M)*, *tet*(O), *tet*(Q), or *tet*(W)

Table 2. Sources of *N. gonorrhoeae* Strains

TP ID	SUPPLIER	SUPPLIER ID	Year Isolated
NG646	ATCC	49226	QC strain
NG648	Jerse Lab	FA1090	1970s
NG649	Jerse Lab	FA19	1970s
NG650	Jerse Lab	F62	1970s
NG651	Jerse Lab	MS11	1970s
NG652	Jerse Lab	LG2	1991
NG653	Jerse Lab	LG7	1992
NG654	Jerse Lab	LG16	1993
NG655	Jerse Lab	LG21	1994
NG656	Jerse Lab	LG22	1994
NG657	Jerse Lab	LGB1	
NG658	Jerse Lab	LGB13	
NG659	Jerse Lab	LGB31	
NG660	Jerse Lab	LGB44	
NG661	Jerse Lab	LGB52	
NG885	Eurofins	2826710	2005
NG886	Eurofins	2826711	2005
NG887	Eurofins	2826712	2005
NG888	Eurofins	2826713	2006
NG889	Eurofins	2826714	2006
NG890	Eurofins	2826715	

Table 3. CLSI Breakpoints

Antibiotic	CLSI Breakpoint S	I	R	CSLI (QC) NG646 ATCC49226
Tetracycline	≤0.25	0.5-1	≥2	0.25-1
Penicillin	≤0.06	0.12-1	≥2	0.25-1
Ciprofloxacin	≤0.06	0.12-0.5	≥1	0.001-0.008
Ceftriaxone	≤0.5	—	—	0.008-0.03

Table 4. MIC_{50} and MIC_{90} Values

Antibiotic	$MIC_{50/90}$ (µg/mL) MIC_{50}	MIC_{90}
TP-271	0.13	0.5
Tetracycline	1	8
Penicillin	0.5	2
Ciprofloxacin	0.008	>2
Ceftriaxone	≤0.008	0.016

Figure 1. Structure of TP-271

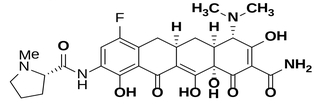

Table 5. Primer Sequences for Detection of Tetracycline-Resistance Genes

Primer Name	Sequence	Reference
16S-F	GCCAGCAGCCGCGGTAATACG	Bradford, *et al*. CMI 14(9) 2008
16S-R	GGACTACCAGGGTATCTAATCC	Bradford, *et al*. CMI 14(9) 2008
TetM-F-sp	AACTCGAACAAGAGGAAAGC	Bradford, *et al*. CMI 14(9) 2008
Tet-M-R-sp	ATGGAAGCCCAGAAAGGAT	Bradford, *et al*. CMI 14(9) 2008
TetA-F	TATGCGTTGATGCAATTTGCCT	this study, GenBank No. AJ419171
TetA-R	CCCACCCGTTCCACGTTGTTATA	this study, GenBank No. AJ419171
ecTetB_F	AATAGTTCGACAAAGATCGCATTGG	this study, GenBank No. AP010961
ecTetB_R	GCACTTGTCTCCTGTTTACTCCCCT	this study, GenBank No. AP010961
Tet-O_F	CACGGAAGCGCTAAAAACAATCTGGGGATT	this study, GenBank No. FJ234438
Tet-O_R	CCTCGCGTTGCATAAATTTCAAAGTGG	this study, GenBank No. FJ234438
Tet-W_F	GCAGCGTGGGATTACCATTCAAGCG	this study, GenBank No. FN825254
Tet-W_R	TCCTCACGCCACCTTTTACGAGGGAGCC	this study, GenBank No. FN825254
TetQ_F	CGACAATGCATCTATTGTAATT	this study, GenBank No. Z21523
TetQ_R	GACGGGTCTTCAATCCACAATG	this study, GenBank No. Z21523
NG-rpsJ_F	TACATCAACACCGGCCGGCAAAT	this study, GenBank No. AE004969 – section 1807359 - 1807670
NG-rpsJ_R	ATGGCAAACCAAAAAATCCGTAT	this study, GenBank No. AE004969 – section 1807359 - 1807670

Conclusions

- TP-271 showed excellent potency against all *N. gonorrhoeae* strains tested (MIC_{90}=0.5 µg/mL) , including those with single and multidrug-resistance phenotypes, and high-level tetracycline resistance.
- TP-271 was active against strains with both ribosome protection (*tet*(M)) and target-based (*rpsJ*) tetracycline-resistance mechanisms.
- If confirmed *in vivo*, these data support TP-271 as a promising new antibiotic for use against infections caused by multidrug-resistant *N. gonorrhoeae*

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These studies were funded in part by NIAID Partnership Grant #: 1R01AI093484 – 01 awarded to CUBRC and Tetraphase Pharmaceuticals;
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