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In Vitro Activity of Fluorocyclines Against *Legionella pneumophila*

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Abstract

Background: TP-434 and TP-271 are new fluorocyclines that are active against all major mechanisms of antibiotic resistance, including tetracycline-specific efflux and ribosomal protection mechanisms. Both are efficacious in murine lung infection models challenged with *Streptococcus pneumoniae* and MRSA. To determine if these compounds could be used as monotherapy for community-acquired bacterial pneumonia, activity against *L. pneumophila* isolates was investigated.
Methods: The *in vitro* activities of TP-434 and TP-271 were compared to tetracycline and erythromycin against a total of 70 *L. pneumophila* isolates (serogroup 1 (n=20), 2 (n=10), 3 (n=10), 4 (n=10), 5 (n=10) and 6 (n=10)) by standard agar dilution using buffered yeast extract agar containing BCYE growth supplement (BYE). A pretest to determine if fluorocycline activity was impacted artificially by BCYE supplement or iron was done by testing ATCC isolates of *Staphylococcus aureus* and *Escherichia coli* on BYE, BYE without ferric pyrophosphate (modBYE) and cation-adjusted Mueller-Hinton agar (MH).
Results: Only BYE supported *L. pneumophila* growth. Pilot tests indicated that BYE resulted in a 16- to 64-fold increase in MICs relative to MH for *S. aureus* ATCC29213 and *E. coli* ATCC25922, suggesting that, similar to other tetracycline class antibiotics, the MIC values of TP-434 and TP-271 obtained in BYE for *L. pneumophila* were artificially elevated due to media effects. Regardless, the $MIC_{50/90}$ values of TP-271, TP-434, tetracycline and erythromycin against all *L. pneumophila* strains were 0.25/1, 1/2, 4/8 and 0.25/0.5 mg/L, respectively. Against *L. pneumophila* serogroup 1, usually the most frequently recovered serogroup, the $MIC_{50/90}$ of TP-271, TP-434, tetracycline, and erythromycin was 0.5/1, 0.5/2, 4/8, and 0.25/0.5 mg/L.
Conclusions: TP-434 and TP-271 had excellent activity against *L. pneumophila*, especially as their activity was artificially suppressed in BYE agar.

Introduction

Legionella organisms are often associated with respiratory infections, and Legionella pneumonia results in significant mortality unless it is promptly and effectively treated. In a recent FDA workshop on Clinical Trial Design for Community-Acquired Bacterial Pneumonia (December 9, 2009), the panel voted to include patients with documented *L. pneumophila* in non-inferioriity community-acquired bacterial pneumonia (CABP) trials. Because *L. pneumonphila* can result in an overall case mortality of 15%, it was important to determine its susceptibility to novel fluorocyclines like TP-434 and TP-271. TP-434 is a broad-spectrum parenteral antibiotic that has completed Phase 1 clinical trials and has been shown to be efficacious in murine models of pneumonia by *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus*. TP-271 has a spectrum consistent with its use for CABP and has shown both oral and IV efficacy in murine pneumonia models.

Materials and Methods

Strains and Growth Conditions

- Recent *Legionella pneumophila* strains were isolated from the respiratory tract from 1992 to 2010 and identified by standard methods described by Murray et al. (1). Isolates from six serogroups were tested for a total number of 70 *L. pneumophila*. Buffered Yeast extract (BYE) (with original *Legionella* BCYE Growth supplement) was used as the medium to test *Legionella* strains.
- *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC29213 were tested in a pilot study comparing the activities of antibiotics in Mueller Hinton Broth (MH), standard BYE, and modified BYE ("Mod BYE"; lacking ferric pyrophosphate). Only data for *E. coli* is shown in Table 1.

Determination of Minimal Inhibitory Concentrations (MICs)

- MICs were determined using the CLSI agar dilution method (2, 3), with replicate plating of the organisms onto a series of agar plates of increasing concentrations of compound from 0.004 mg/L to 64 mg/L.

Results

Table 1. Pilot Media Study: Susceptibility of *E. coli* ATCC 25922 QC Strain

Incubation Time	Media Tested	Antibiotic MIC (mg/L) TP-434	TP-271	Tetracycline	Erythromycin
24 hours	MH	0.12	0.12	1	>64
	Mod BYE	0.5	0.5	0.5	>64
	BYE	2	2	16	>64
48 hours	MH	ND	ND	ND	ND
	Mod BYE	1	0.5	1	>64
	BYE	16	16	>64	>64
Expected MIC range	MH	0.06-0.12*	0.06*	0.5-2**	U

ND= Not Done; U= Unavailable

* Expected MIC Range with Cation-adjusted Mueller-Hinton (CAMHB), Tetraphase in-house data

** Expected MIC Range with CAMHB data obtained from CLSI

Table 2a. Susceptibility of *Legionella pneumophila*

L. pneumophila (no. tested)	Antibiotic	MIC (mg/L) Range	50%	90%
All serogroups (70)	**TP-434**	0.016-2	1	2
	TP-271	≤0.004-2	0.25	1
	Tetracycline	0.5-8	4	8
	Erythromycin	0.06-1	0.25	0.5
serogroup 1 (20)	**TP-434**	0.016-2	0.5	2
	TP-271	≤0.004-2	0.5	1
	Tetracycline	0.5-8	4	8
	Erythromycin	0.06-1	0.25	0.5
serogroup 2 (10)	**TP-434**	0.12-2	1	2
	TP-271	0.3-1	0.25	1
	Tetracycline	1-8	4	8
	Erythromycin	0.06-0.5	0.25	0.25
serogroup 3 (10)	**TP-434**	0.5-2	0.5	2
	TP-271	0.12-2	0.5	1
	Tetracycline	1-8	2	8
	Erythromycin	0.12-0.5	0.25	0.5

Table 2b. Susceptibility of *Legionella pneumophila*

L. pneumophila (no. tested)	Antibiotic	MIC (mg/L) Range	50%	90%
serogroup 4 (10)	**TP-434**	0.25-2	1	2
	TP-271	0.12-1	0.5	0.5
	Tetracycline	2-8	8	8
	Erythromycin	0.12-0.5	0.5	0.5
serogroup 5 (10)	**TP-434**	0.25-1	0.5	1
	TP-271	0.06-0.5	0.25	0.5
	Tetracycline	2-8	4	8
	Erythromycin	0.06-1	0.25	0.5
serogroup 6 (10)	**TP-434**	0.06-1	0.5	1
	TP-271	0.03-0.5	0.12	0.25
	Tetracycline	2-8	4	8
	Erythromycin	0.12-0.25	0.12	0.25

Conclusions

- **TP-434** and **TP-271** are significantly more active than tetracycline against *L. pneumophila*
- Antibacterial activities of **TP-434** and **TP-271** and tetracycline were suppressed in BYE agar
- **TP-434** and **TP-271** are promising agents for the treatment of lower respiratory tract infections caused by *L. pneumophila* serogroups 1 to 6
- See posters F1-2158 and F1-2160 for breadth of spectrum and activity against tetracycline-resistance mechanisms

References

1. Murray et al., Manual of Clinical Microbiology, 9rd ed., 2007, A.S.M. Chap. 53; 835-849.
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3. Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard 17th edition, M7-A7, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, 2006)