

In Vitro Activity of MK-2764 / PTK 0796 Against Legionella spp.

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ABSTRACT

Background
MK-2764 / PTK 0796 is a novel aminomethylene with excellent activity against respiratory pathogens. In order to determine its potential utility in pneumonia, the *in vitro* activity of MK-2764 / PTK 0796 was determined against *Legionella* spp.

Methods

The *in vitro* activity of MK-2764 / PTK 0796 was determined against 15 clinical isolates of *Legionella* spp. by both epidemiology, nucleoside and antinosine. The species tested included *L. pneumophila* serogroup 1 to 12 (25% each), *L. dumoffii* (5), *L. micdadei* (5), *L. longbeachae* (5) and one isolate each of five other species.

Results

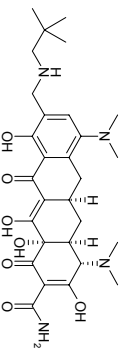
MK-2764 / PTK 0796 (MIC₅₀ 0.25 mg/L) was more active than doxycycline (MIC₅₀ 1 mg/L), azithromycin (MIC₅₀ 0.5 mg/L) and erythromycin (MIC₅₀ 1 mg/L) and was less active than moxifloxacin (MIC₅₀ <0.03 mg/L), gatifloxacin (MIC₅₀ <0.03 mg/L) and rifampicin (MIC₅₀ 0.06 mg/L) against *L. pneumophila*. *L. pneumophila* serogroup 5 had one isolate with the highest MK-2764 / PTK 0796 MIC of 10 mg/L of all the isolates tested. Against other species of *Legionella* spp. MK-2764 / PTK 0796 (MIC₅₀ 0.12 mg/L) and more active than doxycycline and erythromycin (MIC range <0.031 to 0.1 mg/L for both).

Conclusions

These data confirm the interesting activity of the novel aminomethylene antibiotic, MK-2764 / PTK 0796, against *Legionella* spp.

INTRODUCTION

STRUCTURE OF MK-2764 / PTK0796



MK-2764 is a novel aminomethylene antibiotic agent related to tetracycline. Like other tetracyclines, MK-2764 works by inhibiting the normal function of the bacterial ribosome.

In susceptibility studies, MK-2764 is appreciably more potent than most tetracyclines against many Gram-positive pathogens. MK-2764 retains activity against a range of resistant Gram-negative bacilli in the presence of ampicillin, vancomycin and arylalicyclic penicillins like *Legionella pneumophila* and *Chlamydia* spp.

Objective

Determine the minimum inhibitory concentration (MIC) of MK-2764, doxycycline, azithromycin, erythromycin, rifampicin, moxifloxacin and gatifloxacin against a variety of *Legionella* isolated from nosocomial or acquired respiratory tract infections and from environmental sources.

METHODS

Organism and Sources

A variety of *Legionella* was obtained from

- 1) Respiratory tract
- 2) Environmental sources

- Fresh isolates from specimens cultured to produce pure culture
- Multiple cultures from the same patient or source excluded unless a change in organism or antibiotic was noted.
- Origin of organisms done by standard methods such as described by Murray et al, Manual of Clinical Microbiology, 7th ed., 1999, ASM.

Table 1: Tested strains isolated from Respiratory tract or Environmental sources

Micro-organisms	Number of tested strains
<i>Legionella pneumophila</i> (serogroups 1-12)	25*
<i>Legionella micdadei</i>	5
<i>Legionella dumoffii</i>	5
<i>Legionella longbeachae</i>	5
<i>Legionella oakridgensis</i> , <i>Feldia</i> , <i>sanfordensis</i> , <i>Novumitri</i> , <i>germani</i>	5

*7 different serogroups

Procedure for Determinations of MICs

To determine the MIC, a standard *in vitro* doubling concentration broth inoculation method was used using inoculation plates of the organisms onto a series of broth medium microplates of decreasing concentrations was used (NCCLS Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard M7-A6, National Committee for Laboratory Standards, Villanova, PA, January 2003). A series of doubling dilutions were the between 0.003 to 64 mg/L.

- 1) Reagents and materials: Use Buffered Yeast extract (BYE) without divalent as both medium against *Legionella* strains.
- 2) Antibiotic stock solutions: Use antimicrobial concentration diluted in sterile water or deionized solutions for the preparation of stock solutions. Antimicrobial stock solutions prepared at concentrations 1000 mg/L or ten times the highest desired concentration.

- 3) Preparation of double dilution medium microplates: Appropriate dilutions of antimicrobial solution were added to BYE medium. The appropriate dilution of each antimicrobial solution was added to each well of each of the 16 wells containing a final volume of 0.1 ml (40 µl) of broth. The appropriate dilution was added using a dispensing device using antimicrobial dilutions made in at least 10 ml of broth. These dilutions were used to dispense 0.05 (40 µl) ml into each of the 96 wells of a standard tray. Each tray included a growth control well and a negative control well. The inoculum was suspended so that after incubation at 37°C for up to 4 weeks, following the storage, the inoculation trays were allowed to equilibrate to room temperature before use.

4) Interpretation of the inoculation: Test organisms are in the logarithmic phase of growth at the time of exposure to the antibiotic. The MIC is the first well showing no visible growth. The inoculum was suspended so that after incubation at 37°C, the night before each experiment, the standardized inoculum was prepared by according 4-5 colonies of a single type into a tube containing 10 ml of BYE broth. The density of the inoculum was adjusted to a turbidity of McFarland #1 by addition of sterile water. The inoculum was suspended so that after incubation, each well contained approximately 10⁷-10⁸ CFU/well.

5) Incubation of the broth medium microplates: Within 15 minutes after the inoculum prepared, using an inoculating device, a volume of 0.05 ml is added into each well of the BYE broth medium microplate tray containing doubling dilutions of antibiotics. This procedure resulted in a final inoculation of 10⁷-10⁸ CFU/well. The inoculation was performed in a biosafety cabinet at 37°C for 48 hours in aerobic atmosphere. To prevent drying, each tray is sealed with a light-tight plastic cover.

The MICs defined as the lowest concentration of antimicrobial that completely inhibits visible growth. Strains of *Pseudomonas aeruginosa* ATCC27853 and *Legionella pneumophila* ATCC31525 were used as control strains.

RESULTS

Table 2: Susceptibility of *Legionella pneumophila*

Antibiotic	MIC Range (µg/ml) for <i>Legionella</i> Serogroup: 1 (n=8)	MIC Range (µg/ml) for <i>Legionella</i> Serogroup: 3 (n=4)
MK-2764/PTK 0796	0.25	0.25
Doxycycline	1.0	1.0
Azithromycin	<0.03-0.5	<0.03-0.06
Erythromycin	<0.03-0.5	0.12-0.25
Gatifloxacin	<0.03	<0.03
Moxifloxacin	<0.03	<0.03

Table 3: Susceptibility of *Legionella pneumophila* (all serogroups 1, 3, 4, 5 and other serogroups 2, 7 and 9)

Antibiotic	MIC Range (µg/ml) for <i>Legionella</i> Serogroup: 1 (n=8)	MIC Range (µg/ml) for <i>Legionella</i> Serogroup: 3 (n=4)
MK-2764/PTK 0796	0.25	0.25
Doxycycline	1.0	1.0
Azithromycin	<0.03-0.5	<0.03-0.06
Erythromycin	<0.03-0.5	0.12-0.25
Gatifloxacin	0.5	0.5
Moxifloxacin	<0.03	<0.03

Antibiotic	MIC Range (µg/ml) for <i>Legionella</i> Serogroup: 4 (n=6)	MIC Range (µg/ml) for <i>Legionella</i> Serogroup: 5 (n=4)
MK-2764/PTK 0796	0.06-0.25	0.06-1.0
Doxycycline	0.5-1.0	0.5-1.0
Azithromycin	<0.03-0.06	<0.03-0.12
Erythromycin	0.12-0.25	<0.03-0.5
Gatifloxacin	<0.03	<0.03
Moxifloxacin	<0.03	<0.03

Antibiotic	MIC Range (µg/ml) for <i>Legionella</i> Serogroup: 2 (n=3)	MIC Range (µg/ml) for <i>Legionella</i> Serogroup: 7 (n=3)
MK-2764/PTK 0796	0.12-0.5	0.12-0.5
Doxycycline	0.5-1.0	0.5-1.0
Azithromycin	<0.03-0.06	0.06-0.12
Erythromycin	0.5	0.5
Gatifloxacin	<0.03	<0.03
Moxifloxacin	<0.03	<0.03

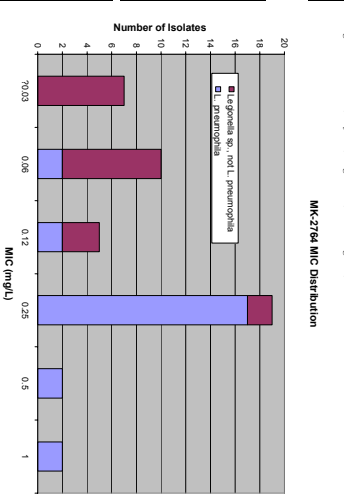
Table 4: Susceptibility of *Legionella* Other than *pneumophila*

Antibiotic	MIC Range (µg/ml) for <i>Legionella</i> species: <i>L. dumoffii</i> (n=5)	MIC Range (µg/ml) for <i>Legionella</i> species: <i>L. micdadei</i> (n=5)
MK-2764/PTK 0796	0.06-0.12	<0.03-0.25
Doxycycline	0.5	0.06-1.0
Azithromycin	<0.03-0.06	<0.03-0.12
Erythromycin	0.12-0.25	0.06-0.25
Gatifloxacin	<0.03	<0.03
Moxifloxacin	<0.03	<0.03

RESULTS

Antibiotic	MIC Range (µg/ml) for <i>Legionella</i> species: <i>L. longbeachae</i> (n=5)	MIC Range (µg/ml) for <i>Legionella</i> species: <i>Other</i> * (n=5)
MK-2764/PTK 0796	<0.03-0.25	<0.03-0.12
Doxycycline	0.06-1.0	<0.03-1.0
Azithromycin	<0.03-0.12	<0.03-0.12
Erythromycin	0.06-0.25	<0.03-0.25
Gatifloxacin	<0.03	<0.03
Moxifloxacin	<0.03	<0.03

* *Legionella bozemanii*, *L. fedtia*, *L. germanii*, *L. oakridgensis*, *L. sanfordensis*



The results of this study indicated that MK-2764 is an effective antimicrobial agent against all *Legionella* species tested. Indeed, the activity of MK-2764 was significantly superior to the standard drugs used for the treatment of Legionnaires disease. The MIC₅₀ of MK-2764 was significantly more active against *Legionella* than MK-2764.

Against *L. pneumophila*, the results from this study indicated that MK-2764 is significantly more active than doxycycline and slightly more active than azithromycin. The activity of MK-2764 against *L. pneumophila* serogroup 1 was reduced from nosocomial and acquired respiratory tract infections. At an MIC₅₀ of 1 mg/L, *L. pneumophila* serogroup 5 were found least susceptible to *L. pneumophila* serogroups.

Against *Legionella* other than *pneumophila*, MK-2764 showed a similar activity. Among these species tested, *L. longbeachae* was the least susceptible to MK-2764. In erythromycin and azithromycin, the activity of MK-2764 was more active against *L. dumoffii*, *micdadei* and other *Legionella* spp. than doxycycline, azithromycin and erythromycin. Against these species, the activity of MK-2764 was similar to the activity of rifampicin.

No difference of susceptibility to MK-2764 was found between the isolates obtained from patients and the isolates obtained from environmental sources.

CONCLUSIONS

MK-2764 should be a promising agent for the treatment of lower respiratory tract infections caused by *Legionella* spp. and clinical studies should be undertaken to evaluate the *in vivo* effectiveness of the new antimicrobial agent.