F1-2040

Evaluation of CEM-101, a Novel Fluoroketolide, in a Rat *H. influenzae* Pulmonary Infection Model

T. M. Murphy1, S. Little1, R. Wu, A. Slee1 , P. Fernandes2 1ViviSource Laboratories, Inc. Waltham, MA 2Cempra Pharmaceuticals, Chapel Hill, NC

Tim Murphy
ViviSource Laboratories, Inc
murphy@vivisource.com
617-401-4603

ABSTRACT

Background: CEM-101, a clinical candidate for the treatment of community-acquired bacterial pneumonia, previously demonstrated significant gram-positive activity in several animal efficacy models. We evaluated the activity of CEM-101 against *H. influenzae*, a gram-negative pathogen, utilizing a difficult-to-treat pulmonary infection model.
Methods: Male Sprague Dawley rats were infected with a macrolide-susceptible or resistant strain of *H. influenzae*. *H. influenzae* was prepared from a plate culture, adjusted to an OD of 0.1 in saline, and diluted 1:2 in 1% molten agar for infection. Anesthetized rats were infected intratracheally with 0.5 mL of the bacterial suspension. Rats were treated with CEM-101, clarithromycin, telithromycin or azithromycin via oral gavage at 5, 24, 48, and 72 hours post-infection. Animals were euthanized 24 or 48 hours after the completion of treatment. The lungs were processed for CFU determination.
Results: CEM-101 demonstrated significant efficacy, achieving 1 and 2 log_{10} reductions in CFUs at 32 and 44 mg/kg when assessed at 24 hours post-treatment. This reduction in bio-load persisted when the time of lung harvest was extended to 48 hours post-treatment. At 48 hours post-treatment, 1 and 2 log_{10} CFU reductions were achieved with 31 and 42 mg/kg of CEM-101. Clarithromycin was unable to elicit a significant reduction in the lung bio-burden levels of *H. influenzae* in this model. Further evaluation with a macrolide resistant strain of *H. influenzae* demonstrated a maximum log_{10} reduction of 1.7 CFU/ gram of lung for CEM-101 when dosed at 75 mg/kg and lung collected at 48 post treatment. Under the same study conditions, clarithromycin only provided a 0.3 log_{10} CFU/ gram of lung reduction and telithromycin demonstrated a 1.4 log_{10} CFU/ gram of lung reduction.
Conclusions: CEM-101 demonstrated significant efficacy in this difficult-to-treat infection model of *H. influenzae* by its ability to effect a bactericidal response. Not only did CEM-101 demonstrate reductions in bio-loads at the classic 24-hour post-treatment harvest assessment, but it also demonstrated significant efficacy when the harvest time was extended to 48 hours post-treatment. Additionally, CEM-101 demonstrated continued activity against a macrolide resistant isolate where clarithromycin provided significantly less bio-burden activity.

INTRODUCTION

CEM-101, a novel fluroketolide antimicrobial agent, has demonstrated substantial activity against susceptible and macrolide resistant bacterial strains (1). Recently, CEM-101 has also demonstrated favorable human pharmacokinetic profiles from Phase I dose escalation studies (4). We have previously reported on the *in vivo* efficacy of CEM-101 against both susceptible and macrolide resistant gram positive isolates including respiratory pathogens such as *S. pneumoniae* (2, 3). *H. influenzae* is a common gram negative pathogen with approximately 3 million infections each year predominantly causing respiratory tract infection. Left untreated the resulting infection can lead to pneumonia and meningitis. Children under the age of five years old are the most susceptible to *H. influenzae* infections (5).
In order to evaluate the effectiveness of CEM-101 against this infecting organism, we utilized a rat lung infection model. In this commonly used infection model we evaluated the activity of CEM-101 against *Haemophilus influenzae* using both a susceptible and a macrolide resistant isolate.

MATERIALS

Antimicrobial agents:
CEM-101, Telithromycin and Clarithromycin powders were provided by Cempra Pharmaceuticals, Chapel Hill, NC.
Azithromycin oral suspension – Henry Schein, Melville, NY
Media:
Chocolate agar plates - BBL, Franklin Lakes, NJ.
Brain Heart Infusion (BHI) Broth - BBL, Franklin Lakes, NJ.
Cyclophosphamide Sigma Aldrich St. Louis MO.

METHODS

Sprague Dawley male rats (weighing approximately 200 grams) from Charles River Laboratories, Inc. (Wilmington, MA) were acclimated for 5 day prior to start of studies. All studies were performed under approved IACUC protocols and conform to OLAW standards. Animals had free access to food and water throughout the study as well as provided enrichment.
In vitro MICs
Minimum inhibitory concentrations for both isolates were performed according to CLSI standards using the broth micro-dilution method.
Rat Pharmacokinetic Assessment:
Rats received a single oral dose of CEM-101 at 20 or 200 mg/kg via oral gavage. At selected time points, blood was collected from three animals per dose concentration. Plasma samples were analyzed by LC/MS for drug level concentrations. Pharmacokinetic parameters (C_{max}, $T_{1/2}$, T_{max}, AUC) were determined through analysis with WinNonLin (Pharsight Corp., Mountain View, CA).
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 625nm of a 1:10 dilution. The adjusted bacterial suspension was mixed 1:2 with 1% molten agar maintained at 42ºC in a water bath. Plate counts were performed to determine actual CFU count.
Rat lung infection model:
Sprague Dawley rats were lightly anesthetized with isoflurane. A volume of 0.5 ml of bacterial inoculum was instilled into the rat lung via intratracheal injection. Animals were observed during recovery from anesthesia and returned to their cages.
Treatment:
Rats received treatment with test article or comparators beginning at 5 hours post infection with three additional treatments delivered at 24, 48 and 72 hours post infection. All compounds were delivered via oral gavage. Infection control animals received dosing vehicle.
Lung Bio-burden assessment:
24 or 48 hours after the last treatment (96 or 120 hours post infection), animals were euthanized and lungs aseptically removed. The lungs were weighed, homogenized to a uniform consistency and serially diluted in saline. Diluted samples were plated on chocolate agar and incubated at 37ºC in 5% CO_2 overnight. The average CFU/ gram of lung were determined.
Neutropenic Rat Lung Infection:
For studies that utilized the macrolide-resistant *H. influenzae* isolate, the rats were rendered neutropenic prior to initiation of the study. Chemical neutropenia was induced with cyclophosphamide at 100 and 75 mg/kg delivered IP on days -4 and -1 respectively.

MICs

Broth microdilution MICs (µg/ml)		
	H. influenzae	
	Susceptible	**Macrolide-resistant**
CEM-101	**1.0**	**1.0**
Telithromycin	1.0	1.0
Clarithromycin	4.0	> 16
Azithromycin	0.5	> 2.0

PHARMACOKINETICS

Pharmacokinetic assessment of CEM-101 in normal rats after a single oral dose

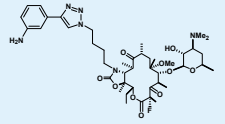
	Plasma	
Parameter	**20 mg/kg**	**200 mg/kg**
Cmax (µg)	0.2	0.9
Tmax (hr)	0.3	2.0
T ½ (hr)	5.4	5.6
AUC (hr µg/mL)	0.4	5.7

PULMONARY INFECTION MODEL

- Susceptible *H. influenzae* isolate
- 24 hour post treatment lung harvest and CFU processing

***H. influenzae* - susceptible**			
		Dose mg/kg	
Compound	Dose route	1 log reduction	2 log reduction
CEM-101	PO	**32**	**44**
Clarithromycin	PO	>50	>50
Azithromycin	PO	28.5	33

CEM-101



PULMONARY INFECTION MODEL

- Susceptible *H. influenzae* isolate
- 48 hour post treatment lung harvest and CFU processing

***H. influenzae* - susceptible**			
		Dose mg/kg	
Compound	Dose route	1 log reduction	2 log reduction
CEM-101	PO	**33**	**42**
Clarithromycin	PO	>50	>50
Azithromycin	PO	23	29
Telithromycin	PO	29	39

- Macrolide resistant *H. influenzae* isolate
- 48 hour post treatment lung harvest and CFU processing

***H. influenzae* - resistant**			
	Route	Dose (mg/Kg)	Log_{10} CFU Change from Controls
CEM-101	PO	75	**-1.7**
Clarithromycin	PO	75	-0.3
Telithromycin	PO	75	-1.4

CONCLUSIONS

CEM-101 is a novel fluroketolide in clinical development by Cempra Pharmaceuticals This compound demonstrates:

- Significant in vitro MICs with a favorable pharmacokinetic profile following oral dosing in rats with a half life of greater than 5 hours.
- Significant 1 and 2 log_{10} reduction values against a susceptible *H. influenzae* in the rat lung model that are equal to or better then known comparators.
- Continued reductions even when time to bio-burden assessment is extended to 48 hours.
- Significant activity against a macrolide-resistant *H. influenzae* isolate with bio-burden assessed at 48 hours post treatment.

REFERENCES

Jones, R. N., Biedenbach, D. J., Rhomberg, P. R., Fritsche, T. R., Sader, H. S. Antimicrobial Characterization of CEM-101 Activity Against 331 Respiratory Tract Pathogens Including Multidrug-Resistant Pneumococcal Serogroup 19A (MDR-19A) Isolates. 48th ICAAC/ 46th IDSA 2008, F1-3975
Murphy, T. M., Gaffney, M., Little. Slee, A. M., Fernandes, P. Evaluation of CEM-101, a Novel Macrolide, in Murine Infection Models. 48th ICAAC/ 46th IDSA 2008, F1-3985
Murphy, T. M., Gaffney, M., Little, S., Wu, R., Slee, A. M., Ong. C., Fernandes, P. Efficacy and Pharmacodynamic Evaluation of CEM-101, a Novel Macrolide, in Murine Infection Models. 19th ECCMID 2009, P-1098.
Still, J. G., Degenhardt, T. P., Scott, D., Fernandes, P., Gutierrez, M. J. Single Oral Dose Pharmacokinetics and Safety of CEM-101 in Healthy Subjects. 48th ICAAC/ 46th IDSA 2008, F1-3972a