

Efficacy and Pharmacodynamic evaluation of CEM-101, a Novel Macrolide, in Murine Infection Models

T. M. Murphy, M. Gaffney, S. Little, R. Wu, A. M. Slee, C. Ong, P. Fernandes, Ph.D., President and CEO, Cempra Pharmaceuticals, Inc., Chapel Hill, NC 27517

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

Abstract

Objectives: To evaluate the in vivo efficacy of CEM-101 against gram positive pathogens including community associated MRSA.

Methods: Efficacy was evaluated in both a subcutaneous abscess model as well as neutropenic thigh infection model. Abscesses were induced in CD-1 female mice by s.c. injection of *S. pneumoniae* or *S. pyogenes* mixed with cytodex beads, CEM-101 or comparator test articles were administered as a single oral dose two hours post infection with bioburden levels assessed at 48 hours post infection. In addition, the neutropenic thigh infection model was utilized to determine target organ efficacy after a single oral dose. CD-1 female mice were rendered neutropenic with cyclophosphamide pre-treatment. Mice were infected with *S. pneumoniae* (SPN) or *S. aureus* via IM injection into the right thigh. At 1.5 hours post infection, mice received treatment via oral gavage with CEM-101 ranging from 1 to 25 mg/Kg. CFUs/gram of thigh were determined at initiation of treatment and at 24 hour post start of treatment. Subsequently, for a preliminary evaluation of PK-PD relationship, mice, infected with SPN, were treated with 4 doses of CEM-101 fractionated into 1, 2, 3, or 4 doses over a 24 hour period. Single dose plasma PK was also performed.

Results: In the abscess, a 10 mg/Kg QD dose of CEM-101 demonstrated a 2.3 log₁₀ decrease while clarithromycin only achieved a 0.9 log₁₀ reduction from untreated mice against SPN. Similarly, a 2.9 log₁₀ decrease was observed for CEM-101 against *S. pyogenes*; while clarithromycin demonstrated only a 0.59 log₁₀ reduction. In the thigh model, CEM-101 demonstrated efficacy after a single oral dose against both susceptible and MRSA isolates. Evaluation of PK-PD demonstrated concentration dependent killing with increased bacterial reduction for the single oral dose over the fractionated cohorts. The effect of CEM-101 on bacterial burden was combined with free drug concentrations to predict the most likely PK-PD parameter. C_{max}/MIC was the best predictor of in vivo efficacy with an r²=0.83.

Conclusions: CEM-101 demonstrated significant in vivo activity in a subcutaneous abscess and neutropenic thigh infection model. Preliminary PK-PD suggests concentration dependent killing with C_{max}/MIC being the best predictor of efficacy against this isolate.

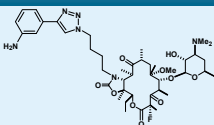
Introduction

CEM-101, a novel fluoroketolide antimicrobial agent, has demonstrated sustained activity against macrolide resistant bacterial strains (2). 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 (3). In these current studies, we evaluated CEM-101 against resistant isolates utilizing tissue bio-burden infection models. The subcutaneous abscess model was employed to assess the ability of CEM-101 to effect a bacterial reduction in a subcutaneous abscess created in immunocompetent mice. For this model we used strains of *S. pneumoniae* and *S. pyogenes*.

To determine the effectiveness of CEM-101 to elicit an antimicrobial response in a model resembling a skin and soft tissue infection, we utilized the neutropenic mouse infection model. We evaluated CEM-101 against macrolide resistant *S. pneumoniae*, as well as community associated MRSA isolates.

We further evaluated the efficacy of CEM-101 by performing pharmacodynamic studies in the neutropenic thigh model and incorporated the pharmacokinetic parameters to establish a preliminary PK-PD relationship for CEM-101.

CEM-101



Materials & Methods

Antimicrobial agents:

•CEM-101, Telithromycin and Clarithromycin powders were provided by Cempra Pharmaceuticals, Chapel Hill, NC.

•Azithromycin oral suspension – Henry Schein, Melville, NY

Media:

•Trypticase Soy Agar (TSA) plates - BBL, Franklin Lakes, NJ.

•Trypticase Soy Agar with 5% sheep blood (TSA-II) - BBL, Franklin Lakes, NJ.

•Brain Heart Infusion (BHI) Broth - BBL, Franklin Lakes, NJ.

•Type III Hog Gastric Mucin - Sigma Aldrich, St Louis, MO

•Cyclodextran Beads - Sigma-Aldrich, St. Louis, MO.

•Cyclophosphamide Sigma-Aldrich, St. Louis, MO.

Methods and Experimental Design

CD-1 Female mice (weighing 18 to 22 grams) from Charles River Laboratories (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.

Mouse Pharmacokinetic Assessment:

Normal CD-1 mice received a single oral dose of CEM-101 at concentrations coinciding with the total doses given in the neutropenic mouse thigh infection model. Blood was collected from 3 animals at selected time points. 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).

In vitro MICs

Minimum inhibitory concentrations of all isolates were performed according to CLSI standards using the broth micro-dilution method.

Mouse Subcutaneous Abscess:

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 cyclodextran beads prepared as per package instructions. The right flank of the mice were shaved and injected subcutaneously with 0.2 ml of the bacterial inoculum. Two hours post infection mice were treated via oral gavage with either test article or control drug. 48 hours post infection, mice were euthanized, abscesses aseptically removed, homogenized, serially diluted and plated on bacterial growth agar. After overnight incubation, colonies were counted and CFUs/gram of abscess were determined.

Neutropenic Mouse Thigh Infection:

Mice were rendered neutropenic by IP injections of cyclophosphamide at day -4 and day -1 of 150 mg/Kg and 100 mg/Kg, respectively. On day 0 mice were infected with approximately 5x10⁵ CFU/ml of bacteria in a 0.1 ml volume into the right thigh. At 1.5 hours post infection mice received treatment via oral gavage. 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 log₁₀ reductions from 24 hour control thighs were calculated. Additional studies were performed that fractionated the single treatment dose (Q24) into two (Q12), three (Q8) and four (Q6) equivalent doses to determine the pharmacodynamic nature of this compound. Further analysis includes static dose, EC₅₀, 1 log kill and maximal effect (Emax).

Minimum Inhibitory Concentrations

	Broth microdilution MICs (µg/mL)			
	CEM-101	Clarithromycin	Telithromycin	Azithromycin
<i>S. pyogenes</i> ATCC 8668	0.008	0.008	0.008	0.008
<i>S. pneumoniae</i> ATCC 6303	0.03	0.03	0.03	0.03
<i>S. pneumoniae</i> #1629	0.03	0.03	0.03	0.03
<i>S. pneumoniae</i> #7701 (mef A)	0.03	0.5	0.03	0.06
MRSA-USA300	0.12	>32	0.25	ND
Community associated				

ND – not determined

Pharmacokinetics

Mouse Plasma PK of CEM-101 after a single oral dose

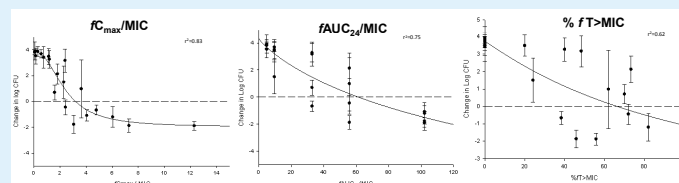
Dose (mg/kg)	Route	T _{max} (h)	C _{max} (ng/ml)	AUC _{0-24h} (ng*hr/mL)	Half-life (h)
2.5	PO	2	163.3	819.22	2.36
5.0	PO	1	550.00	1567.25	1.39
10	PO	2.0	1860.00	9724.16	2.66
25	PO	2.0	3563.33	30826.86	4.36

Neutropenic Thigh Model

MRSA USA300 – Community associated				
Compound	Dose route	Dose mg/Kg QD		
		1 log reduction	2 log reduction	3 log reduction
CEM-101	PO	17.5	27.0	35.5
Telithromycin	PO	42.0	52.5	>60
Clarithromycin	PO	>60	>60	>60

<i>S. pneumoniae</i> #1629				
Compound	Dose Route	Dose (mg/Kg) QD, PO		
		1 log ₁₀ Reduction	2 log ₁₀ Reduction	3 log ₁₀ Reduction
CEM-101	PO	6.0	7.0	8.0
Telithromycin	PO	11.0	13.2	15.5
Clarithromycin	PO	4.5	9.0	13.5

<i>S. pneumoniae</i> #7701 – mef A				
Compound	Dose route	Dose mg/Kg QD		
		1 log reduction	2 log reduction	3 log reduction
CEM-101	PO	10	38	59
Clarithromycin	PO	41	>100	>100
Azithromycin	PO	>100	>100	>100



S. pneumoniae ATCC 6303

	Q24	Q12	Q8	Q6
Static dose (mg/Kg)	8.2	19.2	12.1	20.5
EC ₅₀ (mg/Kg)	5.7	14.5	9.0	16.7
1 log kill (mg/Kg)	10.8	24.0	23.0	23.0
Emax (log ₁₀ CFU thigh)	6.38	4.95	5.01	5.83

Conclusions

CEM 101 is a novel fluoroketolide currently in clinical development This compound demonstrates:

- >Very favorable pharmacokinetic profile following oral dosing in mice.
- >Significant bioad reductions in the subcutaneous abscess and thigh infection models, CEM 101 consistently was more potent than telithromycin, clarithromycin, and azithromycin. These reductions continue even when time to bio-burden assessment is extended to 48 hours.
- >Preliminary PK-PD assessment suggests that C_{max}/MIC to be the best predictor of efficacy.

References

- Bunce, C., Wheeler, L., Reed, G., Musser, J., Barg, N. Murine Model of Cutaneous Infection in Gram-Positive Cocci. Infection and Immunity, 1992, Vol. 60, No. 7.
- Jones, R. N., Biedenbach, D. J., Rhombone, 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, S., Slee, A. M., Fernandes, P. Evaluation of CEM-101, a Novel Macrolide, in Murine Infection Models. 48th ICAAC/ 46th IDSA 2008, F1-3985
- Still, J. G., Deegenhart, 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
- Zak, O., Sande, M. A. Handbook of Animal Models of Infection: Murine Thigh Infection Model Chapter 15. pgs. 137 – 144. Academic Press 1999