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Evaluation of Solithromycin (CEM-101), a Novel Fluoroketolide, in Murine Infection Models

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Abstract

Background: Solithromycin (CEM-101) has demonstrated significant activity against gram positive pathogens including *L. monocytogenes*, *E. faecalis* and marcolide resistant strains of *S. pneumoniae*.
Methods: Efficacy was evaluated in an acute systemic infection model. CD-1 female mice were infected IP; CEM-101 or comparators were administered as a single oral dose 1 hr post infection. PD_{50}s were determined at 48 hr post infection. *L. monocytogenes* was delivered IV with treatment at 1 hr post infection. PD_{50} determined at 72 hr post infection. CEM-101 was evaluated in a cyclophosphamide induced neutropenic mouse model of pneumonia infection. At 5, 24, and 36 hrs post lung infection with a *mef*(E), *erm*(B) resistant *S. pneumoniae* isolate, mice were orally dosed with CEM-101 or control drugs. Twenty-four hr after the end of treatment, the lungs were processed and CFU/gram of lung determined.
Results:

Mouse Systemic Infection Model PD_{50} (mg/kg)			
	CEM -101	Telithromycin	Clarithromycin
S. pneumoniae Serotype 19A (erythromycin R)	**20.6 (15.7–25.4)**	>30	>30
S. pneumoniae mef(E) *& erm*(B)	**21.2 (12.9–29.4)**	> 30	> 30
E. faecalis (macrolide susceptible)	**11.5 (8.5-14.5)**	10.9 (5.0-16.7)	21.8 (16.7-26.8)

In the systemic infection model, *L. monocytogenes* demonstrated PD_{50} values for CEM-101, ampicillin, and azithromycin of 7.6, 55.1, and 11.6 mg/kg, respectively. Reduction in bio-load was evaluated against a resistant *S. pneumoniae* isolate in a neutropenic mouse pneumonia model. CEM-101 achieved 1, 2, and 3 $\log_{10}$ reductions from controls at 30, 46.5 and 85 mg/kg, respectively. Telithromycin achieved a 1 and 2 $\log_{10}$ reductions at 46 and > 100 mg/kg, respectively. Clarithromycin and Azithromycin were unable to effect a significant reduction in bio-load.
Conclusions: CEM-101 has continued to demonstrate significant *in vivo* activity against susceptible and resistant bacteria strains.

Introduction

CEM-101, a novel fluroketolide antimicrobial agent, has demonstrated substantial activity against both susceptible and macrolide resistant bacterial strains *in vitro* susceptibility testing (1, 4, 7). 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* (5, 6). In these series of studies we continue to challenge CEM-101 with additional macrolide resistant isolates in both systemic and tissue burden models. In order to further challenge the activity of CEM-101 we have evaluated clinically relevant strains such as *S. pneumoniae* serotype 19 isolate as well as a macrolide resistant isolate with a *mef* and *erm* resistant genotype (2). CEM-101 has also demonstrated significant *in vitro* activity against intracellular *L. monocytogenes* and selected strains of *E. faecalis* (3).

Materials

Antimicrobial agents:
CEM-101, Telithromycin and Clarithromycin powders were provided by Cempra Pharmaceuticals, Chapel Hill, NC.
Azithromycin oral suspension – Henry Schein Veterinarian Supply, Melville, NY.
Ampicillin – Sigma Aldrich St. Louis, MO.

Media:
TSA-II agar plates - BBL, Franklin Lakes, NJ.
Brain Heart Infusion (BHI) Broth - BBL, Franklin Lakes, NJ.
Cyclophosphamide - Sigma Aldrich St. Louis, MO.

Methods

Female mice, CD-1 or Balb/C, purchased from Charles River Laboratories, Inc. (Wilmington, MA) were acclimatized for 5 days prior to the start of studies. All studies were performed under approved IACUC protocols and conformed to OLAW standards. Animals had free access to food and water throughout the study and were provided enrichment.

***In vitro* MICs:**
Minimum inhibitory concentrations for both isolates were performed according to CLSI standards using the broth micro-dilution method.
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. For systemic infection studies, bacteria were diluted either in BHI broth or hog gastric mucin for injection. For lung infection studies, bacterial inocula were prepared in saline for administration. Plate counts were performed to determine actual CFU counts.
Mouse Systemic Infection Studies:
Mice received treatment via oral gavage 1 hour post IP infection. At termination of the study, percent survival was calculated and the dose effecting 50% survival, the protective dose 50% (PD_{50}), was reported along with 95% confidence intervals as calculated by Probit analysis using GraphPad Prism version 4.03 (GraphPad Software).
For studies involving *L. monocytogenes*, bacteria were administered as an IV injection followed by treatment one hour post infection. Survival was assessed at 72 hours and a Probit analysis performed.
Neutropenia induction:
For the lung infection studies, Balb/C mice were rendered neutropenic through two consecutive IP injections of cyclophosphamide of 150 and 100 mg/kg on days -4 and -1, respectively.
Mouse Lung Infection:
Mice, under light anesthesia, were inoculated with 50 µL of the prepared bacterial inoculum via intranasal inhalation. Mice received treatment via oral gavage at 5, 24, and 36 hours post infection. 24 hours after the end of treatment the mice were euthanized, the lungs aseptically removed, homogenized, serially diluted and plated on bacterial growth agar. After overnight incubation, colonies were counted and CFUs/g of lung were determined.

MICs

Broth microdilution MICs (µg/mL)				
	S. pneumoniae		*E. faecalis*	*L. monocytogenes*
	Serotype 19A; ery R	Mef(E) & Erm(B)		
CEM-101	**0.008**	**0.008**	**0.008**	**0.008**
Telithromycin	0.008	0.03	0.008	0.008
Clarithromycin	> 8	> 32	0.5	N/D
Azithromycin	> 8	> 8	2.0	0.03
Ampicillin	N/D	N/D	N/D	0.06

N/D – Not Determined

Systemic Infection Model

Mouse Systemic Infection Model PD_{50} (mg/kg)			
	CEM-101	Telithromycin	Clarithromycin
S. pneumoniae Serotype 19A (erythromycin R)	**20.6 (15.7–25.4)**	>30	>30
S. pneumoniae mef(E) *& erm*(B)	**21.2 (12.9–29.4)**	> 30	> 30
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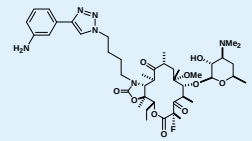
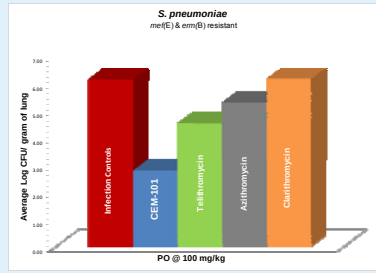
Mouse Systemic Infection Model PD_{50} (mg/kg)			
L. monocytogenes ATCC19111			
	Route	**PD_{50} (mg/kg)**	**95% C.I.**
CEM-101	PO	7.6	5.6 – 9.5
Ampicillin	SC	55.1	25.9 – 84.3
Azithromycin	PO	11.6	8.3 – 14.8

CEM-101



Pulmonary Infection Model

S. pneumoniae **– mef(E) & erm(B) resistant**				
		Dose (mg/kg)		
Compound	Dose route	1 log reduction	2 log reduction	3 log reduction
CEM-101	PO	**30**	**46.5**	**85**
Telithromycin	PO	46	>100	>100
Clarithromycin	PO	> 100	> 100	>100
Azithromycin	PO	> 100	>100	> 100



Conclusions

CEM-101 is a novel fluroketolide currently in clinical development.
This compound demonstrates:

- Significant *in vitro* MICs against susceptible and macrolide resistant isolates.
- Increased *in vivo* activity over telithromycin and clarithromycin against resistant *S. pneumoniae* isolates in systemic infections.
- Equal to or better activity against *E. faecalis* and *L. monocytogenes* in systemic infection models.
- Potent activity against a resistant *S. pneumoniae* isolate in a lung bio-burden efficacy model.

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