Evaluation of CEM-101, a Novel Macrolide, in Murine Infection Models

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Abstract

Background: CEM-101 has demonstrated significant activity against gram + pathogens including macrolide resistant streptococci and MRSA.
Method: Efficacy was evaluated in several infection models. 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 24 hr post infection. CEM-101 was further evaluated in a subcutaneous abscess mouse model against *S. pneumoniae*. CD-1 female mice were infected via SC injection of bacteria mixed with cyclodextran beads. Two hr post infection, mice received a single oral dose of CEM 101 or control agents. At 48 hr post dose, mice were euthanized, abscesses aseptically removed and bacteria enumerated. CFU per abscess was determined and compared to the untreated control. Further evaluation of CEM-101 was performed in cyclophosphamide induced neutropenic mice. At 1.5 hr post thigh infection with *S. pneumoniae*, mice were orally dosed with CEM-101 or control drugs. 24 hr post treatment, the thighs were processed and CFU/ gram of thigh determined.

Mouse Systemic Infection Model (mg/Kg)

	CEM-101	Telithromycin	Clarithromycin
S. aureus	16.3 (11.2-23.3)	>30	22.7 (21.3-34.1)
MRSA	7.5 (6.0-9.9)	ND	5.0
MRSA 300 (CA)	9.5 (5.0-13.9)	9.2 (6.3-12.1)	19.5 (8.2-30.5)
S. pneumoniae (macrolide susceptible)	≤0.03	≤0.06	32.1 (12.3-52.0)
S. pneumoniae (mef R)	≤0.03	10.6 (2.6-18.6)	>30
S. pneumoniae serotype 19A	6.6 (4.4-8.9)	5.7 (4.8-6.7)	5.03 (4.8-5.3)
S. pyogenes (macrolide susceptible)	0.015	7.8 (5.7-9.8)	24.8 (18.1-30.4)
S. pyogenes (erythromycin R)	1.0	4.4 (3.8-4.9)	22.8 (14.6-30.9)

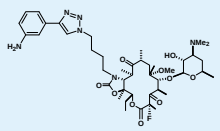
ND = not determined

In the abscess, a 10 mg/Kg QD dose of CEM-101 demonstrated a 4.2 log_{10} decrease while clarithromycin (CL) only achieved a 1.5 log_{10} reduction from untreated mice. CEM-101 in the thigh required 8.0 mg/Kg to achieve a 3 log_{10} reduction from the untreated mice. Telithromycin and CL required 15.5 and 13.5 mg/Kg respectively to achieve the same log_{10} CFU reductions.
Conclusions: CEM-101 demonstrates significant *in vivo* activity in a wide range of infection models.

Introduction

Macrolides have been important members in the antimicrobial armentarium. However, their value has been reduced in recent years due to a dramatic increase in resistance development. Recently, a new class entry, CEM101 being developed by Cempra Pharmaceuticals, has shown excellent *in vitro* and *in vivo* activity against selected macrolide resistant bacteria.
In the studies described herein, we demonstrate very good activity against macrolide sensitive and resistant gram positive bacterial isolates. CEM 101 also shows very favorable oral pharmacokinetics with an extended plasma half life.
Antimicrobial activity was assessed in a murine systemic infection model against several bacterial strains including resistant isolates such as MRSA, MRSA 300, *mef* R *S. pneumoniae*, an erythromycin resistant *S. pyogenes*, and a serotype 19A *S. pneumoniae* isolate.
The ability of CEM101 to reduce the microbial load in abscess and lung infection models was also assessed against *S pneumoniae* isolates.
Further, as a prelude to developing PK PD relationships, the efficacy of oral CEM 101 was assessed in neutropenic thigh infection model using *S pneumoniae*.

CEM-101



Materials and Methods

Antimicrobial agents:
CEM-101, Telithromycin and Clarithromycin powders were provided by Cempra Pharmaceuticals Chapel Hill, NC

Media:
Trypicase Soy Agar (TSA) plates - BBL, Franklin Lakes, NJ.
Trypicase 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.

Materials and Methods cont.

Experimental Design
CD-1 Female mice (weighing 18 to22 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 Systemic Infection Studies:
Eight bacterial isolates were evaluated in this model. For each strain, an overnight culture was utilized. The bacteria were re-suspended in media and diluted either in BHI, 5% or 8% hog gastric mucin to a concentration that would result in 0% survival in mice by 48 hours post infection as determined by initial virulence studies. Bacterial counts were performed to determine inoculum size. Mice received treatment via oral gavage 1 hour post infection. At termination of study, percent survival was calculated and the dose effecting 50% survival, the protective dose 50% (PD50), was reported along with 95% confidence intervals as calculated by Probit analysis using GraphPad Prism version 4.03 (GraphPad Software).

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 shaven 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.

Mouse Lung Infection
Bacteria were prepared from an overnight plate culture by re-suspending in saline and adjusting the suspension to a 0.1 OD at 625 nm of a 1:10 dilution. Mice, under light anesthesia, were inoculated with 50 ul of the bacterial inoculum via intranasal inhalation. Mice received treatment via oral gavage at 5, 24, and 36 hours post infection. 48 hours post end of treatment, mice were euthanized, lungs aseptically removed, homogenized, serially diluted and plated on bacterial growth agar. After overnight incubation, colonies were counted and CFUs/ gram of lung were determined.

Neutropenic Mouse Thigh Infection:
Mice were rendered neutropenic with IP injections of cyclophosamide 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 bacteria 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).

Pharmacokinetics

Mouse Pharmacokinetics of CEM-101

Dose (mg/kg)	Route	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0-24\infty}$ (ng*hr/mL)	Half-life (h)
2.5	PO	1	359.15	1172.63	2.85
10.0	PO	0.5	1436.60	4757.93	2.85

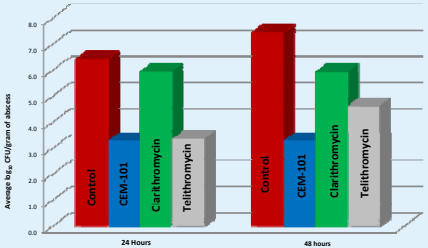
Mouse Systemic Infections

	CEM-101		Telithromycin		Clarithromycin	
	MIC (µg/ml)	PD_{50} (mg/Kg; 95% CI))	MIC (µg/ml)	PD_{50} (mg/Kg; 95% CI))	MIC (µg/ml)	PD_{50} (mg/Kg; 95% CI))
S. aureus	**0.12**	**16.3** (11.2-33.3)	0.06	>30	>16	22.7 (21.3-34.1)
MRSA	**0.12**	**7.5** (6.0-9.9)	0.5	ND	>16	5.0
MRSA 300 (CA)	**0.12**	**9.5** (5.0-13.9)	0.25	9.2 (6.3-12.1)	>16	19.5 (8.2-30.5)
S. pneumoniae (macrolide susceptible)	**≤0.03**	**6.0** (2.0-10.0)	≤0.06	19.9 (9.6-30.2)	>16	32.1 (12.3-52.0)
S. pneumoniae (*mef* R)	**≤0.03**	**23.2** (15.4-38.7)	0.25	10.6 (2.6-18.6)	0.5	>30
S. pneumoniae serotype 19A	**0.25**	**6.6** (4.4-8.9)	0.5	5.7 (4.8-6.7)	>16	5.03 (4.8-5.3)
S. pyogenes (macrolide susceptible)	**0.015**	**9.4** (7.3-11.5)	0.015	7.8 (5.7-9.8)	0.015	24.8 (18.1-30.4)
S. pyogenes (erythromycin R)	**1.0**	**5.1** (4.2-6.1)	1.0	4.4 (3.8-4.9)	1.0	22.8 (14.6-30.9)

ND = not determined

Subcutaneous Abscess Model

Mouse Subcutaneous Abscess ~ *S. pneumoniae* 1629
10 mg/Kg PO @ 2 hours post infection



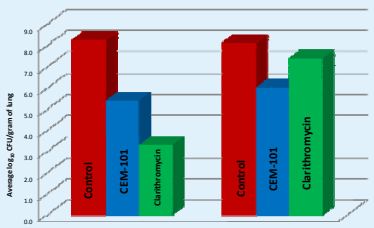
Mouse Subcutaneous Abscess ~ *S. pyogenes* ATCC 8668

	Average Log_{10} CFU/ gram ofAbscess	
	Dose (mg/Kg)	48 hr.
Control	-	8.22
CEM-101	10	5.35
	20	2.53
Clarithromycin	10	7.63
	20	7.82
Telithromycin	10	7.06
	20	6.10

Abscess processing 48 hours post infection

Mouse Lung Infection Model

Mouse Lung Infection – *S. pneumoniae* 1629
CEM-101, Clarithromycin PO @ 5, 24, 36 hr. post infection



Neutropenic Thigh Model

Mouse Neutropenic Thigh *S. pneumoniae* 1629

		Dose (mg/Kg) QD, PO		
		Reduction from 24 hour controls		
Compound	Dose Route	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

Mouse Neutropenic Thigh *S. pneumoniae* 6303

		Dose (mg/Kg) QD, PO		
		Reduction from 24 hour controls		
Compound	Dose Route	1 log_{10} Reduction	2 log_{10} Reduction	3 log_{10} Reduction
CEM-101	**PO**	**1.2**	**3.0**	**5.0**
Telithromycin	PO	4.75	6.5	8.75
Clarithromycin	PO	5.5	9.2	14.0

Mouse Neutropenic thigh Model Fractionated dosing studies
S. pneumoniae 6303 vs. CEM-101 PO

	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 macrolide currently in clinical development
This compound demonstrates:
- Very favorable pharmacokinetic profile following oral dosing in mice.
- In systemic mouse infection studies CEM 101 shows very good activity against *S aureus* MRSA and both macrolide resistant and sensitive *S pneumoniae* and *S pyogenes* isolates with activity comparable or better than telithromycin.
- In models to assess bioload reductions in the subcutaneous abscess, lung and thigh infection models, CEM 101 consistently was more potent than telithromycin and clarithromycin.
- In addition, when the time between the last dose of compound was extended, the bioload reduction levels remained the same, indicating a bactericidal response. By contrast, telithromycin and clarithromycin dose groups demonstrated bioload increases when the time interval was extended. These latter two macrolide/ketolide agents demonstrated a more classical bacteriostatic response.
- The antimicrobial activity in selected murine models of infection support the clinical evaluation of this new member of the macrolide class of antimicrobial agents.

References

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