

Assessment of In Vivo Activity of CEM-102 (Fusidic Acid) in Murine Infection Models

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ABSTRACT

Background: CEM-102 (fusidic acid) is a fusidane antibiotic that demonstrates favorable human pharmacokinetics after oral administration as well as potent MICs against *S. aureus* and MRSA. However, CEM-102 presents a challenge in rodent models due to the very short half-life it exhibits in these species. We have evaluated CEM-102 utilizing a TID dosing strategy to overcome the limited exposure observed in rodent models.

Methods: Systemic infection studies were performed in CD-1 female mice infected IP with *S. aureus* ATCC 13709 and a MRSA USA 300 isolate. Mice received CEM-102 or comparator either as an IV or SC dose at 1, 2, and 3 hours post infection. Survival was assessed for 48 hours and PD₅₀ values were determined. CEM-102 was also evaluated in cyclophosphamide-induced neutropenic mice. Animals were injected IM with *S. aureus* (SA) or *S. pyogenes* (SP). CEM-102 was delivered IV in three doses. Thighs were processed for SA at 24 hours post-treatment, whereas collection was at 8 hours for SP with CFU/gram of thigh determined.

Results: In systemic infection studies, CEM-102 demonstrated PD₅₀ values of 2.1 mg/kg for the IV treatment and 5.7 mg/kg for the SC treatment against *S. aureus* ATCC 13709. A PD₅₀ of 1.5 mg/kg was achieved against a CA MRSA isolate when delivered intravenously. Reduction in bio-load was evaluated in the neutropenic thigh infection model where CEM-102 achieved 1, 2, and 3 log₁₀ reductions from the controls against SA at 15, 26, and 34 mg/kg. Doses required to achieve 1 and 2 log₁₀ reductions against SP were 18 and 49 mg/kg, respectively.

Conclusions: While CEM-102 has demonstrated favorable pharmacokinetics in humans, the assessment of activity in the rodent has been hindered due to a short half-life of the compound and a high metabolic rate in the mouse. To overcome this obstacle, we used a TID dosing strategy. CEM-102 provided significant protection against SA in systemic infection studies and demonstrated significant bio-load reductions for both SA and SP in the neutropenic thigh model.

INTRODUCTION

CEM-102 is a fusidane antimicrobial agent that in clinical development. CEM-102 has demonstrated significant *in vitro* activity against *S. aureus* and MRSA (1, 4). Additionally CEM-102 has been well tolerated in healthy subject receiving both single and multiple doses (5). Though, CEM-102 has demonstrated favorable *in vitro* pharmacokinetic-pharmacodynamic properties, there is a significant disparity in drug exposure between species (6). Most notably, CEM-102 has demonstrated very limited exposure in rodent species (2). Previous studies have demonstrated that fusidic acid dosed three times at 2 hour intervals has resulted in a small reduction in CFU counts against a susceptible *S. aureus* in the thigh model (3). Because, fusidic acid demonstrates limited oral absorption in the mouse, it is difficult to achieve drug plasma levels that will affect an antimicrobial response (7). Due to the limited exposure and fast elimination of CEM-102, we employed a modified dosing strategy to effectively evaluate CEM-102 in rodent efficacy models. By utilizing this dosing strategy we were able to evaluate CEM-102 in a standard mouse septicemia model, as well as the neutropenic mouse thigh infection model.

MATERIALS

Antimicrobial agents:
CEM-102 powder was provided by Cempra Pharmaceuticals, Chapel Hill, NC. Vancomycin clindamycin, trimethoprim, and sulfamethoxazole, were purchased from Sigma Aldrich, St. Louis, MO.

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

METHODS

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 (SD) or TID (0, 1, 2 hours) intravenous doses of CEM-102 at concentration of 80 mg/kg per dose. 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 Septicemia Model:

The bacteria were re-suspended in medium 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 intravenous or subcutaneous injection at 1, 2, and 3 hours post infection. At study termination, 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).

Neutropenic Mouse Thigh Infection:

Mice were rendered neutropenic with IP injections of cyclophosphamide at day -4 and day -1 of 150 mg/kg and 100 mg/kg, respectively (6). On day 0 mice were infected with approximately 5x10⁸ CFU/ml of bacteria in a 0.1 ml volume into the right thigh. At 1.5, 2.5 and 3.5 hours post infection mice received treatment via intravenous injection. One group of infected mice were euthanized and thigh processed for bacterial titers to serve as T=Rx controls (initiation of treatment). Eight or 24 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 8 or 24 hour control thighs were calculated.

MICs

	Broth microdilution MICs (µg/mL)			
	CEM-102	Vancomycin	Clindamycin	TMP-SMX
<i>S. aureus</i> ATCC 13709	0.03	0.5	0.12	0.5
<i>S. pyogenes</i> ATCC 8668	4.0	0.5	ND	ND
MRSA-USA300	0.03	1.0	ND	ND
Community associated				

ND: not determined

PHARMACOKINETICS

- Mouse Plasma PK of CEM-102 after IV administration of a single dose (SD) or 3 doses (TID)

Dose (mg/kg)	Route	T _{max} (h)	C _{max} (µg/ml)	AUC ₀₋₂₄ (µg*hr/mL)	Half-life (h)
80 (SD)	IV	0.08	158.3	70.2	0.42
80 (TID)	IV	0.25	132.0	187.3	0.72

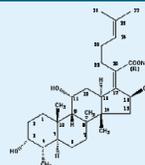
MOUSE SEPTICEMIA MODEL

Mice received 3 doses of each compound at 1, 2 and 3 hours post infection. Survival was assessed at 48 hours post infection.

<i>S. aureus</i> ATCC 13709			
	Route	PD ₅₀ (mg/kg)	C. I.
CEM-102	IV	2.1	1.2 – 2.9
	SC	5.7	3.0 – 8.5
Clindamycin	IV	< 1.0	—
TMP-SMX	SC	0.26	0.13 – 0.39

MRSA CA – USA 300			
	Route	PD ₅₀ (mg/kg)	C. I.
CEM-102	IV	1.5	1.1 – 1.1
Vancomycin	IV	0.81	0.48 – 0.75

CEM-102



NEUTROPENIC THIGH MODEL

Mice received 3 doses at 1, 2 and 3 hours post infection, IV. Thighs were processed for CFUs at 24 hours post treatment.

<i>S. aureus</i> ~ ATCC 13709				
Dose mg/kg QD				
Reduction from 24 hour controls				
Compound	Dose route	1 log reduction	2 log reduction	3 log reduction
CEM-102	IV	15.0	26.0	34.0

Mice received 3 doses at 1, 2 and 3 hours post infection, IV. Thighs were processed for CFUs at 8 hours post treatment

<i>S. pyogenes</i> ~ ATCC 8668				
Dose mg/kg QD				
Reduction from 8 hour controls				
Compound	Dose route	1 log reduction	2 log reduction	
CEM-102	IV	18.0	49.0	

CONCLUSION

CEM 102 is a fusidane antibiotic in clinical development by Cempra Pharmaceuticals. By employing a 3X, IV dosing strategy, we were able to partially overcome the pharmacokinetic deficiency identified when evaluating fusidic acid in the murine model system.

- CEM102 demonstrates:
 - Potent MICs against *S. aureus* and CA-MRSA.
 - Potent activity against a susceptible *S. aureus* isolate and maintains this activity against a MRSA USA 300 (community associated) clinical isolate in septicemia studies.
 - Significant bio-load reductions in the neutropenic thigh infection model against both *S. aureus* and *S. pyogenes* isolates.

Despite the low drug plasma levels and short half life observed in the mouse even after a 240 mg/kg cumulative dose, efficacy against *S. aureus*, CA-MRSA and *S. pyogenes* is still evident.

It is expected that the increased drug exposure with prolonged half-life found in the human could translate to better efficacy against these serious clinical pathogens.

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