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 fusidic 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 post IV with CFU/g 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 an 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 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 fusidic acid potassium salt 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 dissolved 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 attempted to evaluate CEM-102 in rodent efficacy models. By utilizing this dosing strategy we were able to evaluate CEM-102 in a standard mouse sepsis model, as well as the neutropenic mouse thigh infection model.

MATERIALS

Antimicrobial agents: CEM-102 powder was provided by Cempra Pharmaceuticals, Chapel Hill, NC. Vancocin chloride, trimethoprim, and sulfamethoxazole, were purchased from Sigma Aldrich, St. Louis, MO. Media: Tryptic Soy Agar (TSA) plates - BBL, Franklin Lakes, NJ. Tryptic 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 (weighting 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 CLARIT 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 (SO) or TID (0.1, 0.2 hours) intravenous dose of CEM-102 at concentration of 80 mg/kg dose. Blood was collected from 3 animals at selected time points. Plasma samples were analyzed by LC/MS for drug level concentrations. Pharmacokinetic parameters (Cmax, T1/2, Tmax, 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 challenge studies. The bacteria were re-suspended in medium and diluted to result in 0% survival in mice by 48 hours post infection as determined by initial challenge studies. Bacterial counts were performed to determine percent survival. Mice received intravenous (IV) or intraperitoneal (IP) injection of 2.9 x 10^7 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 sacrificed prior to treatment to serve as T0+1 controls (infection initiation). Eight or 24 hours post treatment, the remaining mice were euthanized, thighs aseptically removed, weighed, homogenized, serially diluted and plated on blood agar (Sabouraud). CFUs on 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 6 to 24 hour control thighs were calculated.

MICs

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Inhibition</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEM-102</td>
<td>0.06</td>
<td>0.5</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4.0</td>
<td>8.0</td>
</tr>
<tr>
<td>MBA-102</td>
<td>0.03</td>
<td>0.12</td>
</tr>
</tbody>
</table>

MICs: Dose mg/kg QD

NEUROTROPIC 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. In systemic infection studies, CEM-102 demonstrated significant protection against S. aureus and S. pyogenes isolates. Although 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.

REFERENCES


