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Pharmacokinetics-Pharmacodynamics of CEM-102 (Sodium Fusidate) Against *Streptococcus pyogenes* Using *In Vitro* Pharmacodynamic Models

Brian T. Tsuji[1], Alan Forrest[1,2], Pamela A. Kelchlin[1], Tanya Brown[1], Patricia N. Holden[1], Olanrewaju O. Okusanya[2], Sujata M. Bhavnani[1,2], Prabha Fernandes[3], Paul G. Ambrose[1,2]

[1]University of Buffalo, Buffalo, NY, [2]Institute for Clinical Pharmacodynamics, Latham, NY, [3]Cempra Pharmaceuticals, Chapel Hill, NC

Brian T. Tsuji, Pharm.D.
Dept. of Pharmacy Practice
SUNY, University at Buffalo
Buffalo, NY 14228
Email: btsuji@buffalo.edu

Abstract

Background: The pharmacokinetics-pharmacodynamics (PK-PD) of CEM-102, an oral antibiotic in development for the treatment of complicated skin and skin structure infections, was evaluated for *Streptococcus pyogenes* using *in vitro* pharmacodynamic models (IVPDM).

Methods: Using a 1-compartment *in vitro* pharmacodynamic model (1CIVPM), CEM-102 PK-PD against *S. pyogenes* 991 (MIC=4 mg/L) with an initial 10^6 CFU/mL inoculum was evaluated over 48h. Broth in the IVPDM was supplemented with human albumin (4g/dL). CEM-102 regimens (AUC_{48}:MIC ratio) evaluated included: 600 mg q12h (468), 1200 mg q24h (639), front-loaded (FL)1200-600 mg q12h (922), FL1500-600 mg q12h (1096), 1200 mg q12h (1148) and 2400 mg q24h (1328). The relationship between the log ratio ($AUC_{CFUdrug}/AUC_{CFUControl}$) and AUC_{48}:MIC was evaluated using a Hill-type model. Select regimens were evaluated over 240h using a hollow fiber infection model (HFIM).

Results: CEM-102 regimens demonstrated the following net changes in log_{10} CFU in the 1CIVPDM at 24h/48h: 600 mg q12h, -2.2/-2.5: 1200 mg q24h, -2.4/-3.5: FL1200-600 mg q12h, -1.9/-3.4; FL1500-600 mg q12h, -2.0/-3.4; 1200 mg q12h, -2.2/-3.2; and 2400 mg q24h, -2.3/-3.4. Log ratios were -2.7, -2.6, -2.7, -2.6, -2.6 and -3.1, respectively. The relationship between log ratio and AUC_{48}:MIC was well described by a Hill-type model (r^2 =0.97). AUC_{48}:MICs (%SE) associated with a 1, 2, and 2.5 decline in the log ratio were 26 (160), 110 (143), and 335 (103), respectively. In the HFIM, net changes in log_{10} CFU at 48h/240h of -2.4/-5.8, -2.5/-5.8 and -2.4/-5.8 and log ratio reductions of -3.5, -3.5 and -3.6 for the 600 mg q12h, FL1200-600 mg q12h and FL1500-600 mg q12h regimens, respectively, were observed. All regimens suppressed resistance development over 240h.

Conclusions: CEM-102 regimens evaluated were effective against *S. pyogenes* as demonstrated by > 2 log_{10} CFU reduction at 48h and suppression of resistance over 10 days.

Introduction

- CEM-102, also known as sodium fusidate or fusidic acid, is an oral antibiotic with activity against *Staphylococcus aureus* and *Streptococcus pyogenes*.
- Sodium fusidate has been used for over four decades in Europe, Canada, and Australia for the treatment of complicated skin and skin structure infections.
- Pharmacokinetic-pharmacodynamic (PK-PD) relationships for CEM-102 against *S. pyogenes* have not been previously characterized.
- Application of *in vitro* pharmacodynamic (PD) models allows for the simulation of human pharmacokinetic (PK) profiles and the evaluation of the effect of different CEM-102 dosing regimens against *S. pyogenes*.

Objectives

- The objectives of these analyses were the following:
  - To evaluate the change in bacterial burden of *S. pyogenes* associated with 6 CEM-102 dosing regimens over 48 hrs and the PK-PD relationship for efficacy of CEM-102 against *S. pyogenes* using *in vitro* pharmacodynamic infection models (IVPDM), and
  - To evaluate the change in bacterial burden and suppression of resistance emergence of *S. pyogenes* associated with selected CEM-102 dosing regimens over 240 hours using a hollow fiber infection model (HFIM).

Materials and Methods

Bacterial Isolates, Susceptibility Testing, Antibiotics and Medium

- *S. pyogenes* 991 (CEM-102 minimum inhibitory concentration (MIC) = 4.0 mg/L) was obtained from JMI Laboratories (North Liberty, IA) and MICs were determined by broth microdilution according to Clinical Laboratory Standards Institute.
- The initial inoculum was 10^6 colony forming units (CFU)/mL.
- CEM-102 analytical grade powder was obtained from Cempra Pharmaceuticals.
- Mueller Hinton Broth (MHB) which was used for both IVPDM and HFIM, was supplemented with calcium, magnesium, and human albumin to a final concentration of 4 g/dL, simulating human physiologic concentrations.

***In Vitro* Pharmacodynamic Infection Model**

- A 270-mL one-compartment glass chamber with multiple ports was used for the IVPDM experiments.
- A peristaltic pump was utilized to continually replace antibiotic-containing medium with freshly supplemented MHB at a rate to simulate a pseudo-apparent half-life for CEM-102 of 14.5 hrs (based on human PK data administered 550 mg of CEM-102).
- The IVPDM experiments were performed in duplicate over 48 hrs with samples for CFU determination serially obtained.
- All dosing regimens were administered in a manner to achieve similar 48-hr concentration-time profiles and area under the concentration-time curve (AUC) values as would be expected in healthy volunteers based on the degree of PK accumulation that was expected over that time period [1].
- The following CEM-102 dosing regimens (ratio of AUC at 48 hrs to MIC (AUC_{48}:MIC)) were simulated:
  - 600 mg twice daily (Q12h) (468),
  - 1200 mg Q12h x 2 doses followed by 600 mg Q12h (922) (front-loaded regimen),
  - 1500 mg Q12h x 2 doses followed by 600 mg Q12h (1096) (front-loaded regimen),
  - 1200 mg Q12h (1148),
  - 1200 mg once daily (Q24h) (639), and
  - 2400 mg Q24h (1328).
- An integrated PK-PD area measure, area under the colony forming unit (CFU) curve (AUC_{CFU}) log ratio, was applied to all CFU data to examine PK-PD relationships for efficacy.
- The AUC_{CFU} was determined for each CEM-102 dosing regimen using the linear trapezoidal method. AUC_{CFU} log ratio was then calculated as the log_{10} of the ratio of the AUC_{CFU} for *S. pyogenes* for each dosing regimen to the AUC_{CFU} of the growth control.
- Given the time-dependent bactericidal activity [2] and the presence of a post-antibiotic effect of CEM-102 against Gram-positive organisms [3], AUC:MIC was considered the PK-PD index likely to be most closely associated with efficacy. As a result, the AUC_{CFU} log ratio was described as a function of the 48-hr AUC:MIC ratio using a Hill-type model.

Hollow Fiber Infection Model

- The HFIM (C3008, Cellulosic cartridge, FiberCell Systems Inc., Frederick, MD, USA) used for the evaluation of the CEM-102 dosing regimens against *S. pyogenes* 991 was a two-compartment model that consisted of bundles of hydrophilic fibers encased within a plastic housing (13 mL) connected to an internal pump and a tubing network.
- A peristaltic pump was utilized to continually replace antibiotic-containing medium with freshly supplemented MHB at a rate to simulate a pseudo-half-life of CEM-102 (14.5 hrs) based on human PK data administered 550 mg of CEM-102.
- HFIM experiments were conducted in duplicate over 240 hrs (10 days) with samples for CFU determination taken daily.

Materials and Methods

Hollow Fiber Infection Model (continued)

- The following CEM-102 dosing regimens were simulated:
  - 600 mg Q12h,
  - 1200 mg Q12h x 2 doses followed by 600 mg Q12h (front-loaded regimen), and
  - 1500 mg Q12h x 2 doses followed by 600 mg Q12h (front-loaded regimen).
- The front-loaded CEM-102 dosing regimens were constructed such that the first few doses were predicted to achieve concentrations well above that which would be achieved by administration of the maintenance dosing regimen alone.

Results

***In Vitro* Pharmacodynamic Infection Model**

- The change in *S. pyogenes* bacterial burden over 48 hrs for all the CEM-102 dosing regimens evaluated in the IVPDM is shown in **Figure 1**.
- The net change in log_{10}CFU at 24 and 48 hrs along with the corresponding AUC_{CFU} log ratio for all the dosing regimens evaluated in the IVPDM are provided in **Table 1**.
- By 48 hrs, all of the CEM-102 dosing regimens evaluated achieved a greater than 2-log_{10} CFU reduction of *S. pyogenes*, with suppression of regrowth over this period.
- As shown by reductions in AUC_{CFU} log ratio (% reduction in AUC_{CFU}) of 2.65 (99.8%) and 2.56 (99.7%) compared to 2.68 (99.8%), no discernable differences in the reduction in AUC_{CFU} log ratio were evident for the dosing regimens of 1200 mg Q12h x 2 doses or 1500 mg x 2 doses followed by 600 mg Q12h compared to 600 mg Q12h, respectively.
- Parameter estimates and standard errors (SE) for the Hill-type model describing the relationship between the 48-hr AUC:MIC ratio and AUC_{CFU} log ratio for *S. pyogenes* are provided in **Table 2**.
- **Figure 2** shows the relationship between the 48-hr AUC:MIC ratio and AUC_{CFU} log ratio for *S. pyogenes*, with a fitted function based on the Hill-type model overlaid. As shown by the agreement between the calculated and fitted AUC_{CFU} log ratio values (r^2 of 0.968), the model fit the data well.

Figure 1. Change in *S. pyogenes* bacterial burden over 48 hrs for the CEM-102 dosing regimens evaluated using the IVPDM

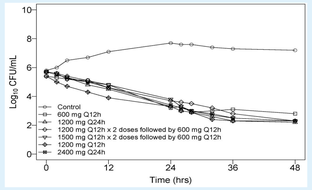


Figure 2. Relationship between the 48-hr AUC:MIC ratio and AUC_{CFU} log ratio for *S. pyogenes*, with a fitted function based on the Hill-type model overlaid

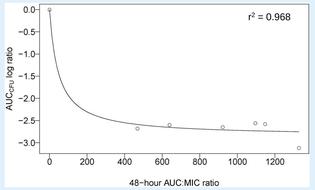


Table 1. The net change in log_{10}CFU at 24 and 48 hrs and corresponding AUC_{CFU} log ratio for all the dosing regimens evaluated in the IVPDM

Dosing Regimen	Net change in log_{10}CFU		AUC_{CFU} log ratio
	24-h	48-h	
600 mg Q12h	-2.2	-2.5	-2.68
1200 mg Q12 x 2 doses followed by 600 mg Q12h	-1.9	-3.4	-2.65
1500 mg Q12h x 2 doses followed by 600 mg Q12h	-2.0	-3.4	-2.56
1200 mg Q12h	-2.2	-3.2	-2.58
1200 mg Q24h	-2.4	-3.5	-2.60
2400 m gQ24h	-2.3	-3.4	-3.12

Results

Table 2. Parameter estimates and SE for the Hill-type model describing the relationship between the CEM-102 48-hr AUC:MIC ratio and AUC_{CFU} log ratio for *S. pyogenes*

Parameter	Parameter estimate	SE
E_{con}	< 0.001	0.226
E_{max}	2.85	0.360
Hill	1.0[a]	Fixed
EC_{50}	47.3	82.2

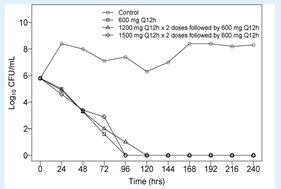
a. H did not differ from 1 when evaluated using Akaike's Information Criteria [4].
E_{con} is the observed effect at zero drug concentration; E_{max} is maximum change in AUC_{CFU} log ratio after 48 hrs; Hill is the sigmoidicity coefficient; EC_{50} is the 48-hr AUC:MIC ratio for which there is 50% maximal change in the AUC_{CFU} log ratio.

- Using the Hill-type model, 48-hr AUC:MIC ratios (%SE) associated with a 1-, 2-, and 2.5-log reduction in AUC_{cfu} log ratio were determined to be 25.5 (160), 110 (143), and 335 (103, respectively.

Hollow Fiber Infection Model

- The change in *S. pyogenes* bacterial burden over 240 hrs for all the CEM-102 dosing regimens evaluated in the HFIM is shown in **Figure 3**.
- Net changes in log_{10} CFU at 48h and 240h of -2.4 and -5.8, -2.5 and -5.8, -2.4 and -5.8, and log ratio reductions of -3.5, -3.5 and -3.6 were observed for the 600 mg q12h, FL1200-600 mg q12h and FL1500-600 mg q12h regimens, respectively.
- Rapid reduction of bacterial counts (99.9% (3-log_{10}) reduction in CFU/mL) was achieved within 72 hrs for all CEM-102 dosing regimens evaluated; complete eradication of bacteria was achieved after 120 hrs for all dosing regimens.
- All of the CEM-102 dosing regimens evaluated suppressed the emergence of all resistant subpopulations, as no bacterial growth was observed on 4, 8, and 16 x MIC drug-containing agar over the 10 day study period (data not shown).

Figure 3. Change in *S. pyogenes* bacterial burden over 240 hrs for the CEM-102 dosing regimens evaluated using the HFIM



Conclusions

- All of the CEM-102 dosing regimens evaluated using the IVPDM achieved a greater than 2-log_{10} CFU reduction of *S. pyogenes*. The PK-PD evaluation of CEM-102 against *S. pyogenes* using an IVPDM allowed for the characterization of the 48-hr AUC:MIC ratios associated with efficacy.
- Data from the HFIM demonstrated complete eradication of *S. pyogenes* after 120 hr with suppression of resistant subpopulations over 240 hr for all CEM-102 dosing regimens evaluated.
- The potent activity demonstrated through these experiments is consistent with the lack of clinical resistance of *S. pyogenes* to CEM-102 and the clinical efficacy of CEM-101 against *S. pyogenes* observed over the last several decades.

References

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