

Poster
 # A1-1933

Using an *in vitro* Pharmacodynamic Model and Mechanism-Based Modeling

B. T. Tsuji¹, J. B. Bullitta², A. Forrest^{1,2}, P. A. Kelchlin¹, T. Brown¹, P. N. Holden¹, M. P. Pai², S. M. Bhavnani^{1,2}, P. Fernandes³, R. N. Jones⁴, P. G. Ambrose^{1,2}
¹SUNY at Buffalo, Buffalo, NY, ²Institute for Clinical Pharmacodynamics/Ordway Research Institute, Latham, NY, ³Cempra Pharmaceuticals, Chapel Hill, NC, ⁴JMI Laboratories, North Liberty, IA

Abstract

Background: The pharmacokinetic-pharmacodynamic (PK-PD) profile of CEM-102, an oral antibiotic in development for the treatment of complicated skin and skin structure infections, was investigated against methicillin-resistant *Staphylococcus aureus* (MRSA) using an *in vitro* pharmacodynamic (PD) model (VPM) and mechanism-based (MB) modeling.

Methods: Using an VPM, the PK-PD of CEM-102 against MRSA USA300 (MIC=0.25 mg/L), at an initial inoculum (CFU_i) of 10⁸ CFU/mL, was evaluated over 48h. Broth was supplemented with human albumin (4g/dL). CEM-102 regimens in mg (AUC_{0-24h}/MIC) included: 550 mg q12h (7,000), 1100 mg q24h (8,030), front-loaded (FL) 550 mg q12h (10,418), 1100 mg q24h (24,397) and FL 1100 mg q12h (32,582). MB models were developed in NONMEM VI, fitting all data simultaneously.

Results: CEM-102 regimens displayed the following net changes in log₁₀ CFU/mL at 24h [48h]: 550 q12h: -0.95 [1.79], 1100 q24h: -1.28 [0.51], FL-550 q12h: -1.13 [-1.07], 1100 q12h: -1.54 [-1.77], 2200 q24h: -1.85 [-1.56] and FL-1100 q12h: -1.65 [-1.67]. The log₁₀ of the area under the CFU/mL curve (AUCFU_{0-24h})/[AUCFU_{0-24h}/MIC] for these regimens was -1.11, -2.03, -2.49, -2.72, -2.61 and -2.62, respectively. An AUC_{0-24h}/MIC ratio of 9433 was predictive of a >2.5 log decline in this area ratio. The MB model included a susceptible and a resistant subpopulation (10⁻⁴ of CFU_i). CEM-102 prolonged the mean generation time up to 3.7-fold with an IC₅₀ of 5.45 mg/L for both subpopulations. CEM-102 also inhibited the probability of successful doubling by up to 66%. Data fits were unbiased and precise for all regimens (slope = 1.00; intercept = -0.02; r = 0.97 for population fit vs. observed log CFU/mL).

Conclusions: Bacterial killing by CEM-102 correlated well with AUC_{0-24h}/MIC ratio. The MB model, which yielded excellent fits of the data, will be useful for further evaluations to support dose selection.

Background

- The treatment of *Staphylococcus aureus* has become a therapeutic problem. The rapidly increasing incidence of community-associated methicillin-resistant *S. aureus* (MRSA), the emergence of highly virulent MRSA strains, and the development of resistance to vancomycin highlight the need for the development of new antimicrobials against MRSA.
- CEM-102, also known as sodium fusidate, is an oral antibiotic with activity against all types of *S. aureus*, including MRSA.
- Sodium fusidate has been used for over two decades in Europe, Canada, and Australia for the treatment of skin and skin-structure infections (SSSI), for which *S. aureus* is a primary pathogen.¹
- For CEM-102, optimal pharmacokinetic-pharmacodynamic (PK-PD) targets have not been elucidated and the bacterial killing profile of CEM-102 has not been fully characterized.
- Application of *in vitro* pharmacodynamic (PD) models allows for the simulation of human pharmacokinetic (PK) profiles. Mechanism-based (MB) PK-PD models constructed using data from such models provide further insight into the dynamic profile of CEM-102, which is the focus of the current investigation

Objectives

- The objectives of these analyses were the following:
- To characterize bacterial reduction profiles of CEM-102 in an *in vitro* PD model.
- To identify optimal PK-PD targets of CEM-102 against *S. aureus*
- To develop a MB PK-PD model that can describe and predict the killing of CEM-102 and aid in development of optimal dosage regimens.

Methods

Bacterial Isolates

- MRSA USA300 (MIC=0.25 mg/L) was obtained from JMI laboratories.
- USA300 is a highly virulent strain of MRSA and is the most common community-associated MRSA strain in the US.

Antibiotics and Medium

- CEM-102 analytical grade powder was obtained from Cempra Pharmaceuticals.
- Mueller Hinton Broth (MHB) was supplemented with calcium, magnesium, and human albumin (SMHB) to a final concentration of 4 g/dL, simulating human physiologic concentrations, for *in vitro* model simulations.

Susceptibility Testing

- MICs were determined by broth microdilution according to Clinical Laboratory Standards Institute in quadruplicate. The results were read after 24hrs of incubation at 37 °C.

In Vitro Pharmacodynamic Model

- 250-mL one-compartment glass chamber with multiple ports was placed in a 37 °C water bath, and an overnight culture was diluted in SMHB broth yielding a starting inoculum of approximately 10⁸ cfu/mL.
- A peristaltic pump was utilized to continually replace antibiotic-containing medium with freshly supplemented MHB at a rate to simulate the half-life of CEM-102 (14.5 hrs) based on human PK data.
- Samples were taken at 0, 2, 4, 8, 12, 24, 26, 28, 32, 36 and 48hrs.
- pH was monitored throughout and ranged from 6.98 to 7.13 for all drug containing regimens experiments.
- All experiments were performed in duplicate.
- The following regimens were simulated (48 hr AUC_{0-24h}/MIC) for CEM-102:
 - 550 mg q12h (7,000)
 - 1100 mg q24h (8,030)
 - Front-loaded: 1100 mg q12h x2 then 550 mg q12h (10,418)
 - 1100 mg q12h (21,327)
 - Front-loaded: 2200 mg q12h x2 then 1100 mg q12h (32,582)
 - 2200 mg q24h (24,397)

Pharmacodynamic Analysis

- The modeled drug effect (E) was the log₁₀ ratio (drug to control) of CFU/mL AUC integrated over 0-48hr (AUCFU_{0-48h}), as is shown in Equation 1.
- A Hill-type model was fit, using non-linear regression, to this Effect (log ratio AUCFU vs AUC_{0-24h}/MIC using Equation 2 where: E₀ is the measured effect at zero drug concentration, E_{max} is the maximal effect, EC₅₀ is the AUC_{0-24h}/MIC for which there is 50% maximal effect, H is the Hill constant.²

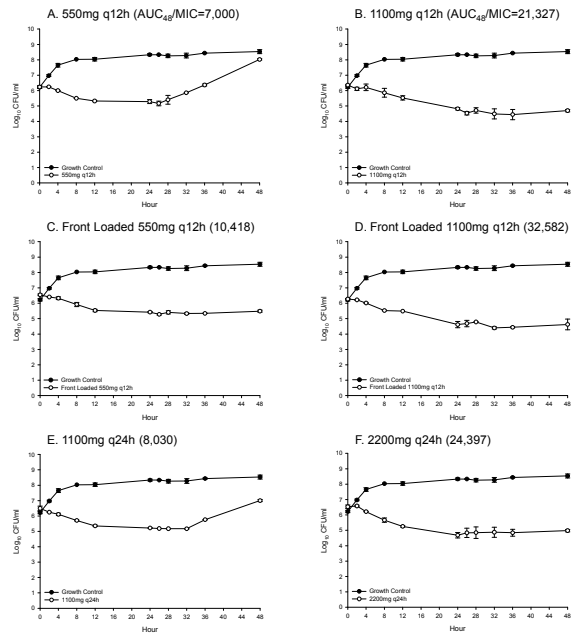
$$(1) E = \log_{10} \left[\frac{AUCFU_{drug}}{AUCFU_{growth\ control}} \right] \quad (2) E = E_0 - \frac{E_{max} \times [AUC_{0-24h} / MIC]^H}{[EC_{50}]^H + [AUC_{0-24h} / MIC]^H}$$

Mechanism-Based PK-PD Models

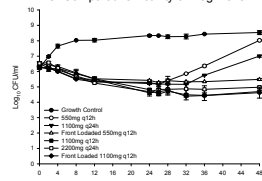
- Model development started with an empirical (initial) model that described the killing by CEM-102 as an inhibition of replication of MRSA.
- Model development of the final MB PK-PD model was based on the misfits of the empirical model.
- The final model was constructed to reflect the mechanism of action of CEM-102.
- All modeling was performed in NONMEM VI (level 1.2) with an additive residual error on log-scale.
- It was assumed that there are two subpopulations, a susceptible and a less susceptible ('resistant') population.³

Results

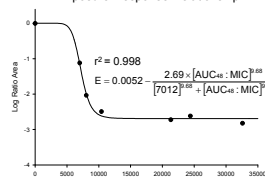
Figure 1. PK-PD of CEM-102 against MRSA USA300 at Varying Dosage Regimens



G. Comparative Activity of Regimens



H. Exposure-Response Relationship



Results

Figure 2. MB PK-PD Model (A) and Model Fitted Parameter Estimates (B)

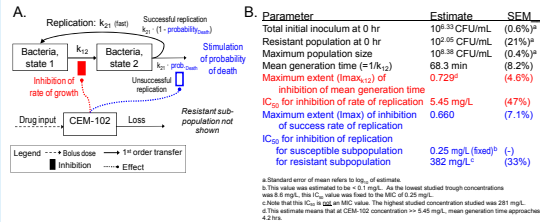


Figure 3. Predicted vs. Observed Plot

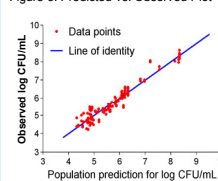
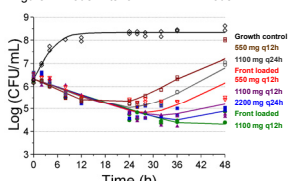


Figure 4. Model Fits for MB PK-PD Model



Conclusions

- A one-compartment model simulating human PK characterized exposures of CEM-102 associated with efficacy.
- The total drug AUC_{0-24h}/MIC ratio for CEM-102 was well correlated with effect.
- Simulated regimens of 550 mg every 12 hrs and 1100 mg every 24 hrs displayed re-growth.
- Front-loading demonstrated a benefit for 550 mg every 12 hrs regimens.
- A PK-PD target was identified for CEM-102 in one-compartment models (AUC_{0-24h}/MIC > 10⁴).
- The MB PK-PD model described the observed log CFU/mL counts for all studied dosage regimens well, performed statistically significantly better than the empirical model, and can account for a slower turnover of bacteria in the presence of CEM-102 in addition to the killing of bacteria.
- Using Monte Carlo simulation and the results from a population PK analysis for CEM-102, the MB PK-PD model described herein will be useful to support dose selection decisions for CEM-102 in future clinical studies.

References

- Howden BP, Grayson ML. Dumb and dumber—the potential waste of a useful antistaphylococcal agent: emerging fusidic acid resistance in *Staphylococcus aureus*. Clin Infect Dis. 2006;42:394-400.
- Tsui JT, et al. Attenuated vancomycin bactericidal activity against *Staphylococcus aureus* hemB mutants expressing the small-colony-variant phenotype. Antimicrob Agents Chemother. 2008;52:1533-7.
- Bullitta JB, et al. Development and qualification of a pharmacodynamic model for the pronounced incoherence effect of ceftazidime against *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2009;53:46-56.
- Bullitta JB, et al. Population pharmacokinetics of CEM-102 in healthy subjects. 49th Interscience Conference on Antimicrobial Agents and Chemotherapy. [A1-1932] San Francisco, CA, September, 2009.