Evaluation of CEM-101, a Novel Fluoroketolide, in a Rat H. influenzae Pulmonary Infection Model

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Background: CEM-101, a clinical candidate for the treatment of community-acquired bacterial pneumonia, previously demonstrated significant gram-positive activity in several animal efficacy models. We evaluated the activity of CEM-101 against H. influenzae, a gram-negative pathogen, utilizing a difficult-to-treat pulmonary infection model.

Methods: Male Sprague Dawley rats were infected with a macrolide-susceptible or resistant strain of H. influenzae. H. influenzae was prepared from a plate culture, adjusted to an OD of 0.1 in saline, and diluted 1:2 in 1% moten agar for infection. Anesthetized rats were infected intra-tracheally with 0.5 mL of the bacterial suspension. Rats were treated with CEM-101, clarithromycin, telithromycin or azithromycin via oral gavage at 5, 24, 48, and 72 hours post-infection. Animals were euthanized 24 or 48 hours after the completion of treatment. The lungs were processed for CFU determination.

Results: CEM-101 demonstrated significant efficacy, achieving 1 and 2 log10 reductions in CFUs at 32 and 44 mg/kg when assessed at 24 hours post-treatment. This reduction in bio-load persisted when the time of lung harvest was extended to 48 hours post-treatment. At 48 hours post-treatment, 1 and 2 log10 CFU reductions were achieved with 31 and 42 mg/kg of CEM-101. Clarithromycin was unable to elicit a significant reduction in the lung bio-burden levels of H. influenzae in this model. Further evaluation with a macrolide resistant strain of H. influenzae demonstrated a maximum log10 reduction of 1.7 CFU gram of lung for CEM-101 when dosed at 75 mg/kg and lung collected at 48 post-treatment. Under the same study conditions, clarithromycin only provided a 0.3 log10 CFU gram of lung reduction and telithromycin demonstrated a 1.4 log10 CFU gram of lung reduction.

Conclusions: CEM-101 demonstrated significant efficacy in the difficult-to-treat infection model of H. influenzae by its ability to elicit a bactericidal response. Not only did CEM-101 demonstrate reductions in bio-burds at the classic 24-hour post-treatment assessment, but it also demonstrated significant efficacy when the harvest time was extended to 48 hours post-treatment. Additionally, CEM-101 demonstrated continued activity against a macrolide resistant strain when clarithromycin provided significantly less bio-burden activity.

INTRODUCTION

CEM-101, a novel fluoroketolide antimicrobial agent, has demonstrated substantial activity against susceptible and macrolide resistant bacterial strains (1). Recently, CEM-101 has also demonstrated favorable human pharmacokinetic profiles from Phase I dose escalation studies (4). We have previously reported on the in vivo efficacy of CEM-101 against both susceptible and macrolide resistant gram positive isolates including respiratory pathogens such as S. pneumoeiae (2, 3). H. influenzae is a common gram negative pathogen with approximately 3 million infections each year predominantly causing respiratory tract infection. Left untreated the resulting infection can lead to pneumonia and meningitis. Children under the age of five years old are the most susceptible to H. influenzae infections (5).

In order to evaluate the effectiveness of CEM-101 against this infecting organism, we utilized a rat lung infection model. In this commonly used infection model we evaluated the activity of CEM-101 against Haemophilus influenzae isolates that were both susceptible and a macrolide resistant isolate.

MATERIALS

Antimicrobial agents: CEM-101, Telithromycin and Clarithromycin powders were provided by Cempra Pharmaceuticals, Chapel Hill, NC. Azithromycin oral suspension – Henry Schein, Melville, NY.

Media: Chocolate agar plates - BBL, Franklin Lakes, NJ. Brain Heart Infusion (BHI) Broth - BBL, Franklin Lakes, NJ. Cyclosporin Sigma Aldrich St. Louis MO.

METHODS

Bacteria preparation for in vivo infections: Bacteria were prepared from an overnight plate culture by re-suspending in saline and adjusting the suspension to a 0.1 OD at 620nm of a 1:10 dilution. The adjusted bacterial suspension was mixed 1:2 with 1% moten agar maintained at 42°C in a water bath. Plate counts were performed to determine actual CFU count.

Rat lung infection model: Sprague Dawley rats were lightly anesthetized with isoflurane. A volume of 0.5 mL of bacterial inoculum was instilled into the rat lung via intra-tracheal injection. Animals were observed during recovery from anesthesia and returned to their cages.

Treatment: Rats received oral or intraperitoneal gavage of test article or comparators beginning at 5 hours post infection with three additional treatments delivered at 24, 48 and 72 hours post infection. All compounds were delivered via oral gavage. Infection control animals received dosing vehicle.

Lung Bio-burden assessment: 24 or 48 hours after the last treatment (68 or 120 post infection), animals were euthanized and lungs aseptically removed. The lungs were weighed, homogenized to a uniform consistency and serially diluted in saline. Diluted samples were plated on chocolate agar and incubated at 37°C in 5% CO2 overnight. The average CFU/gram of lung were determined.

Neutropenic Rat Lung Infection: For studies that utilized the macrolide-resistant H. influenzae isolate, the rats were rendered neutropenic prior to initiation of the study. Chemical neutropenia was induced with cyclophosphamide at 100 and 75 mg/kg delivered IP on days 4 and 1 respectively.

REFERENCES


CEM-101 is a novel fluoroketolide in clinical development by Cempra Pharmaceuticals.

This compound demonstrates:

• Significant in vitro MICs with a favorable pharmacokinetic profile following oral dosing in rats with a half life of greater than 5 hours.
• Significant 1 and 2 log10 reduction values against a susceptible H. influenzae in the rat lung model that are equal to or better than known comparators.
• Continued reductions even when time to bio-burden assessment is extended to 48 hours post treatment.
• Significant activity against a macrolide-resistant H. influenzae isolate with bio-burden assessed at 48 hours post treatment.