Materials and Methods

S. pneumoniae

Neutropenic Thigh Model

r²=0.75

f

burden

SA300

Introduction

C. pneumoniae

2

Methods & Materials

Antimicrobial agents:

- CEM-101, Telithromycin and Clarithromycin powders were provided by Cempra Pharmaceuticals, Chapel Hill, NC.
- Azithromycin oral suspension – Hygenic, Melville, NY

Media:

- Trypticase Soy Agar (TSA) plates - BBL, Franklin Lakes, NJ.
- TSA 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

CD-1 female mice by s.c. injection of S. pneumoniae or S. pyogenes mixed with cytodex beads, CEM-101 or comparator test articles were administered as single oral dose two hours post infection with bioburden levels assessed at 48 hours post infection. In addition, the neutropenic thigh infection model was utilized to determine target organ efficacy after a single oral dose. CD-1 female mice were rendered neutropenic with cyclophosphamide pre-treatment. Mice were infected with S. pneumoniae (SPN) and S. aureus via IM injection into the right thigh. At 1.5 hours post infection, mice received treatment via oral gavage with CEM-101 ranging from 1 to 25 mg/kg. CFU/s thigh were determined at initiation of treatment and at 24 hour post start of treatment. Subsequently, for a preliminary evaluation, PK-PD were performed. Mice, infected with SPN, were treated with 4 doses of CEM-101 fractionated into 1, 2, 3, or 4 doses over 24 hour period. Single dose plasma PK was also performed.

Results: In the abscess, a 10 mg/kg dose of CEM-101 demonstrated a 2.3 log₁₀ decrease while clarithromycin only achieved a 0.9 log₁₀ reduction from untreated mice against SPN. Similarly, a 2.9 log₁₀ decrease was observed for CEM-101 against S. pyogenes, while clarithromycin demonstrated only a 0.5 log₁₀ reduction. In the thigh model, CEM-101 demonstrated efficacy after a single oral dose against both susceptible and MRSA isolates. Evaluation of PK-PD demonstrated concentration dependent killing with increased bacterial reduction for the single oral dose over the fractionated cohorts. The effect of CEM-101 on bacterial burden was combined with free drug concentrations to predict the most likely PK-PD parameter. Cmax/MIC was the best predictor of in vivo efficacy with a (2/10)₃. CEM-101 demonstrated significant in vivo activity in a subcutaneous abscess and neutropenic thigh infection model. Preliminary PK-PD suggests concentration dependent killing with Cmax/MIC being the best predictor of efficacy against this isolate.

Materials and Methods

Abstract

Objectives: To evaluate the in vivo efficacy of CEM-101 against gram positive pathogens including community associated MRSA.

Methods: Efficacy was evaluated in both a subcutaneous abscess model as well as neutropenic thigh infection model. Abscesses were induced in CD-1 female mice by s.c. injection of S. pneumoniae or S. pyogenes mixed with cytodex beads. CEM-101 or comparator test articles were administered as single oral dose two hours post infection with bioburden levels assessed at 48 hours post infection. In addition, the neutropenic thigh infection model was utilized to determine target organ efficacy after a single oral dose. CD-1 female mice were rendered neutropenic with cyclophosphamide pre-treatment. Mice were infected with S. pneumoniae (SPN) and S. aureus via IM injection into the right thigh. At 1.5 hours post infection, mice received treatment via oral gavage with CEM-101 ranging from 1 to 25 mg/kg. CFU/s thigh were determined at initiation of treatment and at 24 hour post start of treatment. Subsequently, for a preliminary evaluation, PK-PD were performed. Mice, infected with SPN, were treated with 4 doses of CEM-101 fractionated into 1, 2, 3, or 4 doses over 24 hour period. Single dose plasma PK was also performed.

Methods and Experimental Design

CD-1 Female mice weighing 18 to 22 grams from Charles River Laboratories (Wilmington, MA) were acclimated for 5 days prior to start of studies. All studies were performed using 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:

Dose (mg/kg) Route T₉₀ (h) Cmax (ng/ml) AUC₀₋₂₄ (ng*hr/mL) Half-life (h)

2.5 PO 168.3 810.22 2.36

5.0 PO 350.00 1587.25 1.39

10 PO 2.0 1880.00 9724.18 2.68

25 PO 2.0 3063.33 30626.8 4.36

Conclusions

Mouse Subcutaneous Abscesses:

Bacteria were prepared from an overnight plate culture by re-suspending in saline and adjusting the suspension to a 0.1 OD at 625nm of a 1:10 dilution. The adjusted bacterial suspension was mixed 1:2 with cyclodextran beads prepared as per package instructions. The right flank of the mice were shaved and injected subcutaneously with 0.2 ml of the bacterial inoculum. Two hours post infection mice were treated via oral gavage with either test article or control drug. 48 hours post infection, mice were euthanized, euthanized, abscesses aseptically removed, homogenized, serially diluted and plated on bacterial growth agar. After overnight incubation, colonies were counted and CFU/s grams of abscess were determined.

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. On day 0 mice were infected with approximately 5x10⁵ CFU/ml of bacteria in a 0.1 ml volume into the right thigh. At 1.5 hours post infection mice received treatment via oral gavage. One group of infected mice was treated with oral gavage and tissue homogenized for bacterial titers to serve as T₉₀ controls. Twenty-four hours post treatment, the remaining mice were euthanized, abscesses aseptically removed, weighed, homogenized, serially diluted and plated on bacterial growth agars. After overnight incubation, colonies were counted and CFU/s were determined.

Mechanistic studies on the neutropenic thigh model and incorporated the pharmacokinetic parameters to establish a preliminary PK-PD relationship for CEM-101.

CEM-101: a novel fuoroketolide antimicrobial agent, has demonstrated sustained activity against macrolide resistant bacterial strains (2). 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 MRSA isolates. In vitro MICs for all isolates were performed according to CLSI standards using the broth micro-dilution method.

In vitro MICs

Minimum inhibitory concentrations of all isolates were performed according to CLSI standards using the broth micro-dilution method.

Mouse PK-PD suggests concentration dependent killing with Cmax/MIC being the best predictor of efficacy against this isolate.

Conclusions

CEM-101 is a novel fuoroketolide currently in clinical development. This compound demonstrates:
- Very favorable pharmacokinetic profile following oral dosing in mice.
- Significant biological reductions in the subcutaneous abscess and thigh infection models. CEM-101 demonstrated more potent activity against clathromycin, clarithromycin, and azithromycin. These reductions continue even when time to bio-burden assessment is extended to 48 hours.
- Preliminary PK-PD assessment suggests that Cmax/MIC to be the best predictor of efficacy.

References


R2 = 0.95

References