Antimicrobial Activity of CEM-101 a New Macrolide, Tested Against Diverse Collections of Bacterial Biowarfare/Bioterrorism Agents

F1-3980

HS Heine, L Miller, J Bassett, K. Holman,
U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD

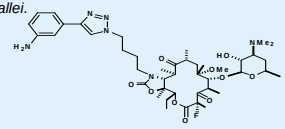
CONTACT:
Division Bacteriology
1425 Porter St
Fort Detrick, MD 21702-501
Phone: 301-619-4887
FAX: 301-619-8351
henry.heine@amedd.army.mil

Abstract

Background: There continues to be a feared scenario of terrorist attacks with aerosolized microorganisms leading to mass infections. Given the added possibility of resistance to current treatments through genetic engineering or natural emergence, identifying effective antibiotics with novel mechanisms of action is critical to counter such an attack. In this study, we determined the minimum inhibitory concentrations (MICs) of a new macrolide CEM-101 against genotypic and geographic diverse collections of five BW/BT agents; *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Burkholderia mallei*, and *B. pseudomallei*.
Methods: Inoculum preparation and antibiotic microdilution were performed according to CLSI methods. MICs for 30 strains of each agent were determined by the microdilution method in 96-well plates, after an 18- or 42-h incubation at 35˚C.
Results: CEM-101, MIC ranges , MIC_{50}, and MIC_{90} (µg/ml) were *B. anthracis* <0.008-0.015, <0.008,<0.008, *Y. pestis* 0.25-2, 1, 2, *F. tularensis* <0.08-4, 0.03, 2, *B. mallei* 0.25-2, 1, 1, and *B. pseudomallei* 16,16,16.
Conclusions: CEM-101 a new macrolide antibiotic had significant *in vitro* activity against many of the biowarfare/bioterrorism (BW/BT) agents tested, with the exception of the *Burkholderia* strains. It has been shown that many macrolides preferentially accumulate intracellularly, which may enhance efficacy when used as a postexposure prophylaxis for preventing pneumonic disease among individuals exposed to aerosolized BW/BT agents. The potential broad-spectrum activity along with oral bioavailability makes CEM-101 an attractive candidate for treating BW/BT exposures and infections. Efficacy of CEM-101 in the animal-infection models for these agents should be evaluated.

Background

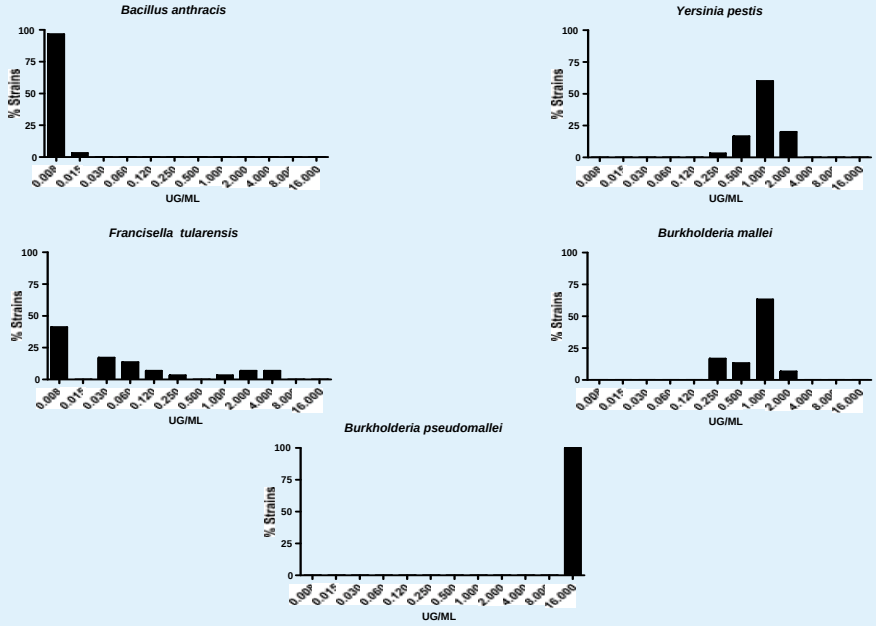
There continues to be a feared scenario of battlefield use or terrorist attacks with aerosolized microorganisms leading to mass infections. Adding the very real possibility of resistance to current treatments through genetic engineering or natural emergence, identifying effective antibiotics which are able to overcome resistance to current approved drugs or have novel mechanisms of action is critical. CEM-101 is a new macrolide that has demonstrated activity against macrolide-resistant bacteria and is more active than azithromycin or clarithromycin against macrolide-susceptible bacteria. In this study, we determined the minimum inhibitory concentrations (MICs) of CEM-101 against genotypic and geographic diverse collections of five biowarfare/bioterrorism agents: *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Burkholderia mallei* and *B. pseudomallei*.



CEM-101 STRUCTURE

Results

CEM-101 Minimum Inhibitory Concentration Distributions



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	# Strains	Range ug/ml	MIC 50 ug/ml	MIC 90 ug/ml
B. anthracis	30	< 0.008 – 0.015	< 0.008	< 0.008
Y. pestis	30	0.25 - 2	1	2
F. tularensis	30	< 0.08 - 4	0.03	2
B. mallei	30	0.25 - 2	1	1
B. pseudomallei	30	16	16	16

Methods

MICs were determined by the microdilution method in 96-well plates according to Clinical and Laboratory Standards Institute (CLSI formally NCCLS) (1). Antibiotics were serially diluted twofold in 50 µl of cation-adjusted Mueller-Hinton broth (CAMHB). For *F. tularensis* determinations CAMHB was supplemented with 2% Isovitalex (Becton Dickinson). The antibiotic range was 16 to 0.008 µg/ml based on a final well volume of 100 µl after inoculation.
Inocula were prepared by suspending colonies from a 18-24 h (*B. anthracis, B. mallei or B. pseudomallei*) or 48 hr (*Y. pestis, F. tularensis*) sheep blood(SBA) or chocolate agar plate (according to CLSI).
Suspended cultures were diluted with CAMHB to a bacterial cell density of 10^6 CFU/ml. To each well of the 96-well plate, 50 µl of this dilution was added for a final inoculum of approximately 5 x 10^4 CFU/well. Plates were incubated at 35ºC. MICs were determined visually at 24- and 48 h and by reading the plates at 600 nm (SpectroMax M2, Molecular Devices).
Thirty strains representing the genetic and geographic diversity of each bacterial species were used in these studies.
Quality control of antibiotic stocks was established by using *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922.

Conclusions

Antibiotic susceptibility testing indicated that all of bacterial species in this study with the exception of *B. pseudomallei* are in therapeutically achievable ranges for CEM-101. *B. pseudomallei* has a demonstrated multi-drug efflux system that is active against macrolides(2) and the data indicating CEM-101 resistance are consistent. The *F. tularensis* distribution reflects overlap of the two distinct biovars “A” and “B”of this species. The B strains distribute to the higher MICs while the A strains distribute to the lower end. A strains are the more virulent and pose the greater BW/BT threat making their greater susceptibility fortuitous.
Since many of these bacterial agents are intracellular during infection the observed ability of many macrolides to preferentially accumulate in cells, may enhance efficacy among individuals exposed to aerosolized BW/BT agents. The demonstrated broad-spectrum activity against a variety of potential BW/BT bacterial agents along with oral bioavailability makes CEM-101 an attractive candidate for treatment after exposures and infections. Efficacy of CEM-101 in the animal-infection models for these agents should be evaluated.

References

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Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Funding Acknowledgement: the research described herein was sponsored by the Defense Threat Reduction Agency, JSTO-CBD program, Project No. TMTI0054-08-RD-T