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In Vitro and *In vivo* Activity of CEM-101, a new Fluoroketolide, Against *Mycobacterium avium* Complex

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ABSTRACT

Background: *M. avium* complex (MAC) infection is a serious health concern for AIDS patients. Standard treatment regimens consist of clarithromycin (CLA), ethambutol and a rifamycin. Treatment failure is often attributed to the emergence of CLA-resistance. A new fluoroketolide, CEM-101, has demonstrated potent activity against several gram postive bacteria. In this study we compare the *in vitro* activity of CEM-101 to CLA against both CLA-susceptible (S) and resistant (R) isolates of MAC as well as comparing the *in vivo* activity against a CLA-S isolate.

Methods:
In vitro: The MICs of CLA and CEM-101 were measured against 12 CLA-S and 12 CLA-R isolates using a microtiter broth dilution assay.
In vivo: Forty-two C57BL/6 mice were infected intranasally with 2.4 X 10^7 CFU of MAC ATCC 49601. Treatment with CLA at 200mg/kg or CEM-101 at 200, 100, 50 or 25mg/kg was started one week post-infection and administered by gavage 5 days/week for 4 weeks. Mice were euthanized at the completion of therapy. Spleens and right lungs were processed to determine the bioburden.

Results: The MICs of CLA against the CLA-S strains ranged from 0.125 to 8µg/ml while the MICs for CEM-101 ranged from 0.125 to 16µg/ml. The MICs of CLA against the CLA-R isolates ranged from 32 to 128µg/ml while the range for CEM-101 was 8 to 16µg/ml. *In vivo* CEM-101 at 200mg/kg was better than CLA at 200mg/kg in both the spleens and lungs. CEM-101 activity was dose dependent.

Conclusions: CEM-101 had potent activity against both CLA-S and CLA-R MAC and demonstrated better activity compared to CLA in the mouse model. Considering the excellent tissue and intracellular distribution, and low metabolism of CEM-101 in man, CEM-101 may have the potential of replacing CLA in treatment regimens against CLA-resistant MAC infection.

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INTRODUCTION

M. avium complex (MAC) infection is a serious health concern for patients living with AIDS, who numbered an estimated 34.4 million people world-wide in 2008. MAC is the causative agent for more than 90% of the non-tuberculous mycobacterial infections in these patients[1]. Standard treatment regimens consist of clarithromycin (CLA), ethambutol and a rifamycin. Treatment failure is often attributed to the emergence of CLA-resistance. A new ketolide, CEM-101, has demonstrated potent activity against several gram postive bacteria including macrolide-resistant pathogens. This study was undertaken to compare the *in vitro* activity of CEM-101 to CLA against both CLA-susceptible and CLA-resistant isolates of MAC as well as comparing the *in vivo* activity against a susceptible isolate of *M. avium* in a murine model of infection.

MATERIAL AND METHODS

In vitro.

Drugs. Clarithromycin (CLA) and CEM-101 were provided by Cempra Pharmaceuticals. Clarithromycin was dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 1mg/ml. CEM-101 was dissolved in water with glacial acetic acid (added until dissolved) at a final concentration of 1mg/ml. CEM-101 was filter sterilized by passage through a 0.22µm-pore sized membrane filter. All drugs were aliquoted and frozen at -20ºC until use.

Isolates. Clinical isolates were obtained from SUNY Upstate Medical University and MAC 101 (ATCC 700898) and MAC LPR (ATCC 49601) were obtained from ATCC (Manassas,VA). The isolates were grown in modified 7H10 broth (pH 6.6; 7H10 agar formulation with agar and malachite green omitted) with 10% OADC (oleic acid, albumin, dextrose, catalase) enrichment (BBL Microbiology Systems, Cockeysville, MD) and 0.05% Tween 80 for 5-10 days on a rotary shaker at 37ºC. The culture was diluted to 10 Klett units (equivalent to 5 X 10^7 colony forming units (CFU)) per ml (Photoelectric Colorimeter; Manostat Corp., New York, NY). The culture was frozen at -70ºC until use. On the day of testing the culture was thawed and diluted to a final concentration of 1.25 X 10^5 CFU/ml. The final inoculum size was determined by titration, in duplicate, on 7H10 agar plates supplemented with 10% OADC enrichment (BBL Microbiology Systems, Cockeysville, MD). The plates were incubated at 37ºC in ambient air for 14 days.

Broth dilution method. Polystyrene 96 well round – bottom microtiter plates (Corning Inc., Corning, NY) were prepared by adding 50µl of modified 7H10 broth with serial dilutions of the drugs to be tested using a multichannel electronic pipetter. To each well 50µl of the appropriate mycobacterial cell suspension was added to yield a final concentration of about 6 x 10^4 CFU/ml (range for various isolates tested was 2.2 X 10^6 CFU/ml – 7.3 X 10^4 CFU/ml). Each drug was tested in duplicate. The microtiter plates were covered with SealPlate adhesive sealing film (Excel Scientific, Wrightwood, CA) and incubated at 37ºC in ambient air for about 5-7 days prior to reading. The MIC was defined as the lowest concentration of antimicrobial agent yielding no visible turbidity.

In vivo.

Mice. Six-week old female C57BL/6 mice were purchased from Jackson Laboratories, Bar Harbor, ME and were maintained within the Syracuse VA Medical Center's Veterinary Medical Unit, Syracuse, NY. All animal procedures were approved by the Subcommittee for Animal Studies (SAS). Mice were housed in micro-isolator cages (lab products inc, Maywood, NJ) and maintained with water and Prolab RMH 3000 rodent chow (Purina, St. Louis, MO).

Drugs. On the day of treatment CLA was dissolved in 20% ethanol followed by 5 minutes of sonication and was dosed at a concentration of 200mg/kg in a 0.2ml volume (actual ethanol concentration was 4%). CEM-101 was dissolved in 0.5% methyl cellulose followed by 5 minutes of sonication and was dosed at 200, 100, 50 or 25mg/kg.

Isolate. *M. avium* LPR ATCC 49601 was grown in modified 7H10 broth (pH 6.6; 7H10 agar formulation with agar and malachite green omitted) with 10% OADC (oleic acid, albumin, dextrose, catalase) enrichment (BBL Microbiology Systems, Cockeysville, MD) and 0.05% Tween 80 for 5-10 days on a rotary shaker at 37ºC. The culture was diluted to 100 Klett units (equivalent to 5 X 10^8 colony forming units (CFU)) per ml (Photoelectric Colorimeter; Manostat Corp., New York, NY). The final inoculum size was determined by titration, in
triplicate, on 7H10 agar plates supplemented with 10% OADC enrichment (BBL Microbiology Systems, Cockeysville, MD). The plates were incubated at 37ºC in ambient air for 14 days.

Infection study. Mice were infected intranasally with 2.4 X 10^7 CFU of M. avium LPR. Mice were randomly assigned to 7 groups of 6 mice: Early Control (EC), Late Control (LC), CLA 200mg/kg, CEM-101 200mg/kg, CEM-101 100mg/kg, CEM-101 50mg/kg, CEM-101 25mg/kg.
One week post-infection mice were treated with the above agents orally by gavage in a 0.2ml volume 5 days/week for 4 weeks. The EC group was euthanized at the initiation of therapy to determine the infection load. An LC group was euthanized at the end of treatment to determine the level of infection without therapy.
Mice were sacrificed by CO_2 inhalation after the completion of therapy. Right lungs and spleens were aseptically removed and ground in a sealed tissue homogenizer (IdeaWorks! Laboratory Devices, Syracuse, NY). The number of viable organisms was determined by serial dilution and titration on 7H10 agar plates. Plates were incubated at 37ºC in ambient air for 14 days prior to counting.

Statistical evalution. The viable cell counts were converted to $\log_{10}$ and then evaluated by ANOVA. Post-hoc analyses were conducted using Tukey's Multiple Comparisons Test. Statistical significance was accepted with P value of less than 0.05.

RESULTS

In vitro (Table 1). The range of MICs for the CLA-S strains against CLA was 0.125 to 8µg/ml while the MICs for CEM-101 ranged from 0.125 to 16µg/ml. The MICs of CLA against the CLA-R isolates ranged from 32 to 128µg/ml while the range for CEM-101 was 8 to 16µg/ml.

Table 1. MIC of Clarithromycin (CLA) and CEM-101 versus M. avium complex.

Isolate	CEM 101 MIC µg/ml	Clarithromycin MIC µg/ml	Clarithromycin Resistant-R or Susceptible-S
Mycobacterium avium			
MAC LPR	8	2	S
MAC 1408	0.25	0.25	S
MAC 9141	1	0.25	S
MAC BMT	0.125	0.25	S
MAC FAR	0.125	0.125	S
MAC KZL	8	1	S
MAC GRL	8	2	S
MAC TRY	8	0.5	S
MAC 101	0.25	0.125	S
MAC KAL	16	8	S
MAC TEL	8	2	S
MAC 3404.4	0.125	0.125	S
MAC 103	8	64	R
MAC Pay	8	64	R
MAC 320	8	64	R
MAC 216	8	32	R
MAC 843	8	64	R
MAC 309	8	64	R
MAC 921	16	128	R
MAC 810	16	64	R
MAC 110	8	64	R
MAC 824	8	128	R
MAC 826	8	64	R
MAC 108	8	64	R

Legend
R-resistant
Control Strain

In vivo (Figure 1 and 2). There was a significant increase in growth of MAC LRP in the spleens and lungs of untreated mice over the 4 week treatment period (P < 0.05). The reduction in growth of MAC LPR in the spleens and lungs was significant for mice receiving CLA at 200mg/kg and CEM-101 at 200, 100 and 50mg/kg compared to the late controls (P < 0.05), but not for CEM-101 at 25mg/kg (P > 0.05). Although the reduction in CFU of MAC LPR was greater in both the spleens and lungs of mice receiving CEM-101 at 200mg/kg compared to the CLA at 200mg/kg group it was not statistically significant (P > 0.05). There was a dose response of CEM-101 observed in both the spleens and lungs.

Figure 1. CFU recovered from the spleens of mice infected with MAC LPR.

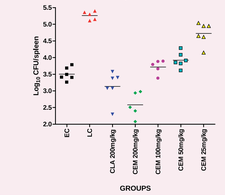
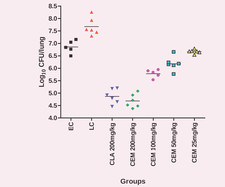


Figure 2. CFU recovered from the lungs of mice infected with MAC LPR.



CONCLUSIONS

The new fluoroketolide CEM-101 had potent *in vitro* activity against both CLA-R and CLA-S MAC isolates. The range of MICs for CEM-101 was unchanged when comparing the activity between the resistant and susceptible strains. The CEM-101 also had potent *in vivo* activity that was slightly better than CLA against the CLA-S *M. avium* strain MAC LPR in the murine model of *M. avium infection*. We utilized the standard model to test the anti-mycobacterial activity of CLA and CEM-101 which includes therapeutic interruptions for 2 days during the weekends[2]. It is not possible to predict the impact of these brief therapeutic interruptions on the efficacy of either CLA or CEM-101. Considering the excellent tissue and intracellular distribution[3], and low metabolism of CEM-101 in man, CEM-101 may have the potential of replacing CLA in treatment regimens against both CLA-susceptible and CLA-resistant MAC infection.

SELECTED REFERENCES

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