Poster 1137

Mycobacterium leprae is susceptible to Solithromycin (CEM-101)

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Abstract

Solithromycin (CEM-101), a new macrolide-ketolide in clinical development, has been found to be a minimum of 4-fold more active than other macrolides, mainly clarithromycin and azithromycin and 2-4 fold more active than Telithromycin. It is active against a variety of macrolide-resistant pathogenic strains of *S. aureus*, *S. pyogenes*, and *S. pneumoniae*. The efficacy of Solithromycin (CEM-101) against *Mycobacterium leprae*, the causative agent for leprosy, was investigated in the present study.

Thai-53 isolate of *M. leprae*, maintained by serial passages in athymic *nu/nu* mice footpads, was used for all experiments. For axenic testing freshly harvested viable *M. leprae* were incubated in medium along with different concentrations of the drugs (CEM-101, clarithromycin and rifampin) for 7 days at 33ºC. At the end of this incubation drug-treated *M. leprae* were subjected to radiorespirometry to assess viability based on oxidation of ^{14}C palmitate. For intracellular testing peritoneal macrophages from Swiss mice were infected with freshly harvested viable *M. leprae* at a MOI of 20:1 for 12 hours. At the end of the infection macrophage cultures were washed free of extracellular bacteria and drugs added at different concentrations and incubated for 3 days at 33ºC. At the end of 3 days cells were lysed to obtain the intracellular *M. leprae* for viability testing by radiorespirometry and staining with viability dyes to assess the extent of membrane damage.

Solithromycin (CEM-101) at 0.15 mg/ml was able to significantly (P<0.001) reduce the viability of *M. leprae* in both axenic and intracellular cultures when compared to controls. Inhibition by CEM-101 was not statistically different from inhibition obtained with clarithromycin under identical conditions and at the same concentration against the claithromycin-susceptible *M. leprae*.

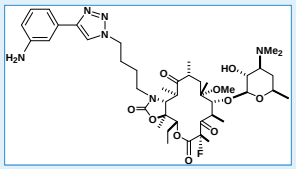
Solithromycin (CEM-101) is effective against *M. leprae* potentially expanding the drugs available to treat leprosy.

Introduction

Solithromycin (CEM-101), a new macrolide-ketolide in clinical development, has been found to be a broad-spectrum antibacterial. Macrolides have had wide therapeutic applications in infectious diseases and have a history of safety and efficacy. Solithromycin has progressed through formal pre-clinical regulatory studies, and advanced into Phase I clinical stage in 2008. It is currently in a phase II study involving community-acquired bacterial pneumonia. Solithromycin has been shown to be active against *M. avium* and intracellular pathogens, such as *Listeria* and *Legionella.* It is available in both oral and intravenous formulations. Here we describe Solithromycin as a potential anti-leprosy drug candidate based on *in vitro* studies.

Mycobacterium leprae the causative agent for leprosy can not be grown *in vitro*, so studying bactericidal or bacteriostatic effect of drugs is problematic. Since there is no direct means to assess bacterial killing, other than mouse foot pad assay, which is extremely cumbersome and time consuming (6-9 months), we relied on two indirect assessments for *M. leprae* viability *in vitro;* radiorespirometry (RR) which measures the rate of palmitic acid oxidation in a suspension of *M. leprae* and viability staining (VS) which scores for membrane damage in individual bacteria. Lower RR values and higher VS scores indicate a drop in *M. leprae* viability *in vitro*.

Chemical Structure of Solithromycin (CEM-101)



Materials and Methods

Nude mouse derived *M. leprae*: The Thai-53 isolate of *Mycobacterium leprae* was maintained in the foot pads of athymic nu/nu mice infected for 4-6 months, and then harvested as described previously (Truman 2001), and enumerated by direct count according to Shepard's method (Shepard 1968). Freshly harvested viable bacilli were always employed in experiments within 24 hr of harvest.

Axenic culture of *M. leprae* with drugs: 1x10^8 *M. leprae* were suspended in 1ml of 7H12 Medium containing different concentrations of drugs (CEM-101, clarithromycin and rifampin) and incubated at 33ºC for 7days. At the end of incubation the viability of *M. leprae* were assessed by radiorespirometry (RR).

Radiorespirometry (RR): Metabolism of suspensions of control and drug treated *M. leprae* was measured by evaluating the oxidation of ^{14}C-palmitic acid to $^{14}CO_2$ by RR as described previously (Franzblau 1988). Captured $^{14}CO_2$ determines the rate of metabolism. In the present study the 7th day cumulative counts per minute (CPM) are reported.

Materials and Methods

Intracellular culture of *M. leprae* with drugs: Medium RPMI-1640, supplemented with 25 mM HEPES (Sigma), 2 mM glutamine (Sigma) and 10% (v/v) FCS was used throughout these studies. Resident peritoneal cells from Swiss mice were harvested and allowed to adhere for at least 6hr at 37ºC and 5% (v/v) CO_2, on plastic cover slips (Miles Laboratory) in 24 well tissue culture plates (Ramasesh 1991). After washing to remove non-adherent cells, the adherent cells were infected overnight at 33ºC with fresh nude mouse foot pad derived *M. leprae* at a multiplicity of infection of 20:1. At the end of the incubation extracellular *M. leprae* were removed by washing the cover slips and different drugs were added at appropriate concentrations. Equal number of cells from each group were lysed with 0.1M NaOH (Sigma) at 72hr, and the intracellular *M. leprae* processed for RR viability staining (VS).

Fluorescent viability staining (VS) of *M. leprae*: The membrane integrity of drug treated *M. leprae* was evaluated with a *LIVE/DEAD BacLight Bacterial Viability Kit*® (Molecular Probes) as described previously (Lahiri 2005). Briefly, *M. leprae* were incubated for 15 min at room temperature with Syto9 and propidium iodide (PI). The bacteria were washed and resuspended in 10% (v/v) glycerol in normal saline. The dead and live bacteria were enumerated by direct counting of fluorescent green and red bacilli using appropriate single bandpass filters. The excitation/emission maxima are 480 nm / 500 nm for Syto9 and 490 nm / 635 nm for PI.

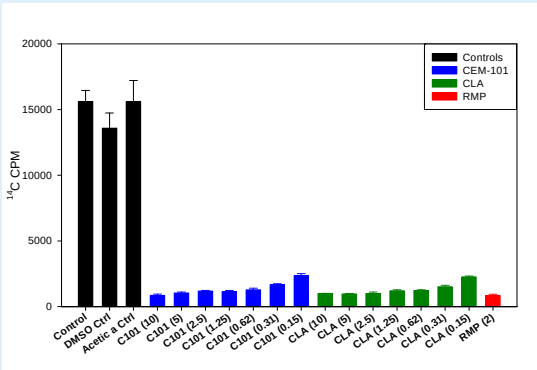
Statistical analysis. The data are shown as mean ± standard deviation (SD) from a representative of three to four experiments. The raw data were subjected to Student's t test to determine whether the observed differences between the means were significant. $P < 0.05$ was taken as significant.

Results

***M. leprae* treated with CEM-101 in axenic medium.**

Freshly harvested athymic nu/nu mice foot pad derived live *M. leprae* were incubated with either CEM-101 or clarithromycin (CLA) serially double diluted from 10μg/ml to 0.15μg/ml in 7H12 medium for 7 days at 33ºC. Rifampin (RMP) was also used at a concentration of 2μg/ml as a positive control. 7th day cumulative RR data (**Fig. 1**) shows that CEM-101 significantly reduced (P<0.001) *M. leprae* palmitic acid oxidation at 0.15μg/ml concentration when compared to no drug controls. But this reduction was not significantly different (P=0.864) when compared to that of CLA at the same dose under identical conditions. The inhibition profile of *M. leprae* palmitic acid oxidation following 7 days incubation was very similar between CEM-101 and CLA over the studied dose range of 10μg/ml to 0.15μg/ml.

Figure 1 – 7th day Radiorespirometry data showing inhibition of *M. leprae* palmitic acid oxidation following 7 days incubation with Solithromycin (CEM-101), Clarithromycin (CLA) and Rifampin (RMP). Numbers within parenthesis indicate the dose in μg/ml.



***M. leprae* treated with CEM-101 in axenic medium.**

Resident peritoneal macrophages were infected with freshly harvested athymic nu/nu mice foot pad derived live *M. leprae* at 20:1 and incubated with either CEM-101 or clarithromycin (CLA) at doses ranging from 10μg/ml to 0.15μg/ml for 3 days at 33ºC. The intracellular *M. leprae* were subjected to both RR and VS post-incubation. RR data (**Fig. 2**) shows that CEM-101 significantly (P<0.001) reduced *M. leprae* metabolism at 5μg/ml when compared to control. Similarly VS data (**Fig. 3**) also show that CEM-101 caused significant membrane damage to intracellular *M. leprae* at this dose level. However there was no difference between the effect of CEM-101 and CLA at 5μg/ml. We did not observe any significant adverse effect of CEM-101 or CLA on intracellular *M. leprae* on doses lower than 5μg/ml (data not shown).

Results

Figure 2 – Radiorespirometry showing inhibition of intracellular *M. leprae* metabolism following 3 days incubation with Solithromycin (CEM-101), Clarithromycin (CLA) and Rifampin (RMP). Numbers within parenthesis indicate the dose in μg/ml.

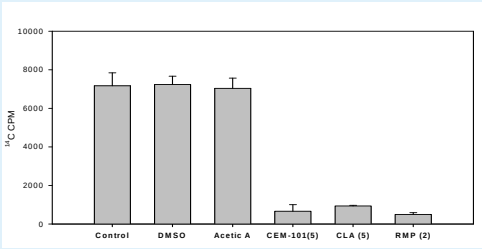
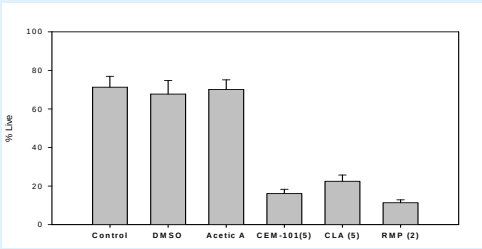


Figure 3 – Viability staining showing percentage of live intracellular *M.leprae* following 3 days incubation with Solithromycin (CEM-101), Clarithromycin (CLA) and Rifampin (RMP). Numbers within parenthesis indicate the dose in μg/ml.



Discussion/Conclusions

- Solithromycin (CEM-101) showed reduced *M. leprae* palmitic acid oxidation at 0.15μg/ml in axenic medium after 7 days of incubation. This is similar to that of Clarithromycin.
- Solithromycin caused reduced metabolism and membrane damage to intracellular *M. leprae* after 3 days of incubation with the drug at a concentration of 5μg/ml.
- Solithromycin may be a candidate for new anti-leprosy drug.

References

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