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. Resistant peritoneal cells from Swiss mice were harvested and allowed to adhere for at least 6 hr at 37°C and 5% (v/v) CO₂ on plastic cover slips (Miles Laboratory) in 24 well tissue culture plates (Ramsayesh 1991). After washing to remove non-adherent cells, the adherent cells were infected overnight at 33°C 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 0.1 M NaOH (Sigma) at 72 hr, and the intracellular M. leprae processed for RR fluorescence 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 intracellular and extracellular bacteria were enumerated by direct counting of fluorescent green and red bacilli using appropriate single bandpass filters. The exclusion/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

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

Shen CM, Dellitello-MI, Stanev MR, Ackerman M, Cynamon MH. In vitro and in vivo activity of CEM-101, a new fluoroketolide, against M. avium complex. 2010. Abstr # E-2057, 50th ICAAC meeting, Boston, MA.