

Solithromycin (CEM-101), a Novel Fluoroketolide with Exceptional Cellular Accumulation, Localizes in Lysosomes and Induces Phospholipidosis but no Apoptosis and does not Interfere with the Production of Reactive Oxygen Species (ROS) in Cultured. Comparison with Azithromycin (AZM) and Gentamicin (GEN)

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Background:

The novel fluoroketolide Solithromycin (CEM-101) accumulates to high levels in cells (AAC 53:3734-43, 2009). Based on previous observations with azithromycin (AZM) and gentamicin (GEN), we have examined whether CEM-101 is sequestered in lysosomes, causes phospholipidosis, induces apoptosis, and interferes with reactive oxygen species (ROS) production.

Methods:

(i) cell fractionation of cells incubated with [¹⁴C]-labeled CEM-101; **(ii)** assay of cell phospholipids and in vitro measurement of inhibition of lysosomal phospholipase A1 (PaseA1); **(iii)** DAPI-detected apoptosis in incubated and electroporated cells; **(iv)** increase in the fluorescence intensity of CM-H₂DCFDA (ROS detection). For incubated cells, CEM-101 and AZM were used at 0-100 mg/L, whereas GEN was used 0-900 mg/L to compensate for its low uptake by cultured cells.

Results:

(i) distribution: [¹⁴C]-labeled CEM-101 co-distributed for 50-70% in lysosomes in J774 macrophages and LLC-PK1 proximal tubular cells, with the remainder in cytosol; **(ii)** total phospholipids: ~1.5-fold increase for fibroblasts incubated 2-3 days with CEM-101 or AZM (10 mg/L) or with GEN (0.9 g/L); IC₅₀ towards PaseA1: CEM-101 and GEN: ~ 50 μM, AZM: ~ 100 μM; **(iii)** Apoptosis (LLC-PK1 cells) (a) incubated: < 5 % with CEM-101 or AZM vs. ~15 % with GEN; (b) electroporated: no change over control for CEM-101 vs. ~15 % for GEN (both 0.3 mM); **(iv)** ROS production: no constant change in H₂DCFDA fluorescence (up to 50 mg/L) for CEM-101 and AZM in LLC-PK1 cells.

Conclusions:

Like AZM and GEN, CEM-101 causes lysosomal phospholipidosis in relation to its accumulation in lysosomes and its inhibitory activity towards PaseA1. This alteration, however, is not associated with apoptosis and causes no constant change in ROS production. This suggests that phospholipidosis induced by CEM-101 is unlikely to cause marked cell toxicity or antimicrobial defense impairment at microbiologically meaningful concentrations.