

Antimicrobial activity of the fluoroketolide solithromycin (CEM-101) against Neisseria gonorrhoeae

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Abstract

Objectives: To evaluate the in vitro activity of solithromycin and its intracellular activity against clinical gonococcal isolates as well as its intracellular activity against isolates highly resistant to azithromycin.

Methods: A total of 196 clinical Neisseria gonorrhoeae isolates collected from 2008 to 2011 at the Public Health Ontario Laboratory, Toronto, Canada, were studied, including isolates previously characterized and a collection of strains with different levels of azithromycin resistance. In vitro activity of solithromycin was compared to azithromycin using the agar dilution method according to the Clinical and Laboratory Standards Institute guidelines. The role of pH in MIC determinations, using pH-adjusted agar plates (pH range, 5.6 to 7.6) was determined. To investigate the intracellular activity of solithromycin, in vitro invasion assays were performed using monolayers of HeLa epithelial cells and five clinical gonococci expressing different azithromycin susceptibility profiles. Infected cultures were treated with solithromycin and its intracellular activity was determined by counting viable intracellular bacteria after 3 and 20 hours of exposure

Results: Solithromycin displayed an MIC₅₀ and an MIC₉₀ of 0.0625 and 0.125 mg/L, respectively, making its activity at least 4-fold higher than azithromycin. Clinical isolates with elevated MICs for azithromycin (MICs of ≥2.048 mg/L, and 4-8 mg/L) showed solithromycin MIC values of 8 mg/L and 0.5mg/L, respectively. In contrast with results obtained with azithromycin, solithromycin MICs were not significantly affected by acidic pHs, suggesting more stability at lower pH. Moreover, when N. gonorrhoeae were internalized by Hela cells and exposed to solithromycin at 4X. 1X and 1/4X the MIC of each strain, our results showed the exposure of infected HeLa cells cultures at 4X the MIC and 1X the MIC resulted in the progressive loss of viability of most of the strains compared to time zero. These data suggest an efficient intracellular activity of the fluoroketolide against a variety of N. gonorrhoeae strains presenting different levels of susceptibility to azithromycin, including isolates highly resistant to the macrolide (e.g. MIC ≥2.048 mg/L).

Conclusion: The stability and the efficient intracellular activity of solithromycin combined with the low MICs of this agent for N. Gonorrhoeae strains make it an attractive option for gonorrhoea treatment, especially when multidrug resistant clinical strains displaying full resistance to azithromycin and to 3rd generation cephalosporins are now emerging.

Introduction and Objectives

Resistance to extended-spectrum cephalosporins (ESC, cefixime and ceftriaxone) has recently emerged in Asia and Europe, threatening their use as first-choice antimicrobials.13 Solithromycin (CEM-101), a novel fluoroketolide, has a reported high potency against Grampositive and negative pathogens.4.8 Golparian et al. have reported that the in vitro activity of solithromycin against clinical gonococcal isolates and international reference strains, including strains with various high-level antimicrobial resistance was superior to that of azithromycin and many other antimicrobials.9

To evaluate the potency of solithromycin for the treatment of gonococcal infections, we investigated its in vitro activity against N. gonorrhoeae strains recently collected in Ontario (Canada) including azithromycin susceptible and resistant isolates. With a subset of selected strains, we also investigated the pH stability and the intracellular activity of solithromycin using a tissue culture model of cervical, epithelial cells,

Materials and Methods

Strains

A total of 196 N. gonorrhoeae clinical isolates were collected from 2008 to 2011. Among these isolates, we included 67 isolates which have been examined in our previous study for their susceptibility profiles (8 antibiotics)10, as well as strains susceptible, with reduced susceptibility. and resistance to azithromycin. Macrolide resistant isolates included in this study were genetically characterized as described.10

Determination of MICs

Each sample was subcultured twice on NYC agar before antimicrobial testing. The MICs of solithromycin and azithromycin were determined using the CLSI agar dilution method11, with replicate plating of the organisms onto a series of agar plates of increasing concentration from 0.015 mg/L to 8 mg/L for solithromycin and from 0.031 mg/L to 2048 mg/L for azithromycin. N. gonorrhoeae strains WHO L (intermediate resistance to azithromycin, 0.5 mg/L) and P (resistant to azithromycin, 2 mg/L) were included as guality control strains.12 EUCAST breakpoints for azithromycin were used: S. ≤0.25 mg/L: I. 0.5 mg/L: R. ≥1 mg/L.

Role of pH in MIC determinations

For susceptibility testing of selected N. gonorrhoeae strains to solithromycin and azithromycin at different pHs. GC agar with pHs ranging from 6.4 to 7.6 were in house-prepared and buffered using 0.1 M potassium phosphate buffers (6.4 to 7.6). The final pH values of the GC agar plates were confirmed using a flat pH surface electrode.

Intracellular activity of solithromycin

Five N. gonorrhoeae clinical strains demonstrating susceptibility (NG48) and resistance (NG640. NG641 NG642 and NG726) to azithromycin (EUCAST breakpoints) were used for all the experiments. HeLa cells were infected at an MOI of 80. After internalization of the bacteria, cells were exposed to solithromycin at 4X. 1X and 1/4X the MIC of each strain. At indicated times, cells were harvested and bacterial viability was determined.

Figure 1. In vitro activity and MIC distribution of solithromycin and azithromycin against N. gonorrhoeae (N=196)



Figure 2. Susceptibility of N. gonorrhoeae isolates to solithromycin and azithromycin in pH-adjusted GC agar



Results



Conclusions

- Solithromycin has superior in vitro antigonococcal activity (lower MIC) against a variety of clinical strains displaying intermediate susceptibility or high level resistance to azithromycin (e.g. MIC ≥2.048 mg/L)
- MIC₅₀ of 0.0625 mg/L and MIC₉₀ of 0.125 mg/L were observed for solithromycin, making its activity 4-fold higher than azithromycin (Figure 1). In the case of one strain with high level azithromycin resistance (MIC ≥2,048 mg/L), a low MIC for solithromycin of 8 mg/L was observed (reduction of at least 8-fold).
- Solithromycin showed stable activity at different pH values against N. conorrhoeae whereas azithromycin showed a marked decrease in potency against all strains from pH 76 to 64

Solithromycin demonstrated efficient intracellular activity against a variety of N. gonorrhoeae strains with different levels of susceptibility to azithromycin, including isolates highly resistant to the macrolide (e.g. MIC ≥2.048 mg/L)

The intracellular activity of solithromycin combined with the low MICs of this agent for N. gonorrhoeae make it an attractive option for treatment of gonococcal infections, especially when multidrug-resistant strains displaying full resistance to azithromycin and to 3rd generation cephalosporins are now emerging clinically.

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Figure 3. Intracellular activity of solithromycin against N. gonorrhoeae clinical strains