

Abstract

The goals of this study are to evaluate the *in vitro* activity of solithromycin, a fluoroketolide, against clinical gonococcal isolates, and test its intra-cellular activity against isolates highly resistant to macrolides.

Clinical isolates (N=196) collected from 2008 to 2011 at the Public Health Ontario Laboratories, Toronto, Canada, were studied, including isolates previously characterized¹ and a collection of strains with different levels of azithromycin (AZI) resistance. MICs of solithromycin and AZI were compared by agar dilution method, and the role of pH into these determinations was defined (pH range, 5.6 to 7.6). Monolayers of HeLa epithelial cells infected with gonococci expressing different AZI susceptibility profiles were treated with solithromycin to test its intracellular activity by bacterial counting after 3 and 20 hours of exposure.

Solithromycin displayed MIC₅₀ and MIC₉₀ of 0.0625 and 0.125 µg/ml, respectively, making its activity at least 4-fold higher than AZI. Clinical isolates with elevated AZI MICs (>2,048 µg/ml, and 4-8µg/ml) showed solithromycin MICs of 8 µg/ml and 0.5µg/ml, respectively. Solithromycin displayed more stability at lower pH since MICs were not significantly affected by acidic pHs, compared to AZI MICs in the same pH conditions. Moreover, the exposure of infected HeLa cells cultures to 1x or 4x the bacterial solithromycin MICs resulted in marked loss of viability of all tested strains, suggesting a very high intracellular antimicrobial activity.

In conclusion, solithromycin demonstrated to be stable and potent against *N. gonorrhoeae*, even on strains with high azithromycin MICs. These extracellular and intracellular results suggest that solithromycin might be an effective treatment option for gonorrhoea if *in vitro* studies combined with clinical trials demonstrate that this drug can be used for this indication.

Introduction

Resistance to extended-spectrum cephalosporins (ESC, cefixime and ceftriaxone) has recently emerged in Asia and Europe, threatening their use as first-choice antimicrobials.¹⁻³

Solithromycin (CEM-101), a novel fluoroketolide, has a reported high potency against gram-positive 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.⁵ Solithromycin has been demonstrated to have good oral bioavailability with good tissue and intracellular distribution. It has been safe and well-tolerated.¹³ It has progressed through Phase 2 in trials in CABP and has shown good efficacy, safety and tolerability in these studies.¹⁴

Objectives

- To evaluate the *in vitro* potency of solithromycin against *N. gonorrhoeae*.
- To investigate the pH stability and the intracellular activity of solithromycin using a tissue culture model.

Materials & Methods

Strains. 196 *N. gonorrhoeae* clinical isolates, collected from 2008 to 2011, were studied, including 67 isolates previously studied¹⁰, 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. The MICs of solithromycin and azithromycin were determined using the CLSI agar dilution method.¹⁴ *N. gonorrhoeae* strains WHO L (intermediate resistance to azithromycin, 0.5 mg/L) and P (resistant to azithromycin, 2 mg/L) were included as quality 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 (pH 6.4 to 7.6).

Intracellular activity of solithromycin. Five *N. gonorrhoeae* clinical strains demonstrating susceptibility (NG48) and resistance (NG640, NG641, NG642 and NG726) to azithromycin 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.

Results

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

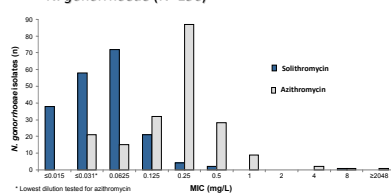


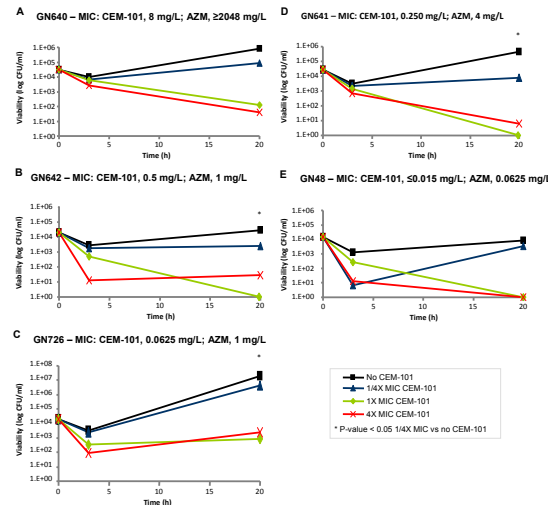
Table 1. Susceptibility of *N. gonorrhoeae* isolates to solithromycin and azithromycin in pH-adjusted GC agar

Strains	MIC (mg/L)	pH			
		6.4	6.8	7.2	7.6
GN640 (AZM ^r)	AZM	2,048	2,048	2,048	2,048
	Solithromycin	4	8	8	8
GN641 (AZM ^r)	AZM	16	16	4	4
	Solithromycin	0.25	0.25	0.25	0.0625
GN723 (AZM ^r)	AZM	64	32	8	4
	Solithromycin	0.5	0.25	0.25	0.25
GN725 (AZM ^r)	AZM	2	1	1	0.5
	Solithromycin	≤0.125	≤0.125	≤0.125	≤0.125
GN726 (AZM ^r)	AZM	2	2	1	1
	Solithromycin	≤0.125	≤0.125	≤0.125	≤0.125
GN733 (AZM ^r)	AZM	4	2	1	0.5
	Solithromycin	≤0.125	≤0.125	≤0.125	≤0.125
GN 11G0 332145 (AZM)	AZM	1	1	0.5	0.5
	Solithromycin	≤0.125	≤0.125	≤0.125	≤0.125
GN 11G0332006 (AZM)	AZM	1	1	0.5	0.25
	Solithromycin	≤0.125	≤0.125	≤0.125	≤0.125

AZM, azithromycin. MIC determinations were performed in pH-adjusted GC plates. EUCAST breakpoints for AZM were used: S, ≤0.25 mg/L; I, 0.5 mg/L; R, ≥1 mg/L.

Results

Figure 2. Intracellular activity of solithromycin against *N. gonorrhoeae*



Conclusions

- Solithromycin has superior *in vitro* antagonococcal 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. In the case of one strain with high level azithromycin resistance, 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 whereas azithromycin showed a marked decrease in potency against all strains from pH 7.6 to 6.4.

- Solithromycin demonstrated efficient intracellular activity against strains with different levels of susceptibility to azithromycin, including isolates highly resistant to the macrolide.
- The intracellular activity of solithromycin combined with the low MICs of this agent for *N. gonorrhoeae* make it a potential option for treatment of gonococcal infections, especially when multidrug-resistant strains are now clinically emerging.
- Additional *in vitro* studies combined with clinical trials need to be performed to demonstrate that this drug can be used for this indication.