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In vitro activity of Solithromycin (CEM-101) among azithromycin resistant and susceptible *Mycoplasma genitalium* strains.

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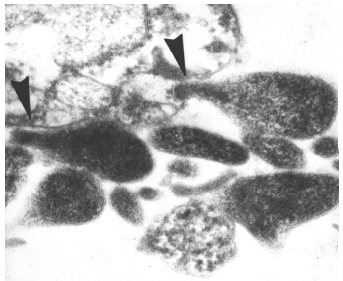
Abstract

Objectives: *Mycoplasma genitalium* is a well-established and important cause of sexually transmitted infections (STIs). Treatment with a 1 g single dose azithromycin as for *Chlamydia trachomatis* infections induces high-level macrolide resistance in the 5-15% of cases where eradication fails. Moxifloxacin is currently the only second-line drug, but multi-drug resistant strains of *M. genitalium* are emerging. In this study, we evaluated the in-vitro activity of the new fluoroketolide solithromycin (CEM-101), and compared it to other antimicrobials currently used for treatment of *M. genitalium* infections.
Methods: The minimum inhibitory concentrations (MIC) of solithromycin, azithromycin, erythromycin, doxycycline, ciprofloxacin, and moxifloxacin were determined for a collection of 40 *M. genitalium* isolates originating from 38 patients. MICs were determined using a cell culture method where a defined inoculum (2500 geq by quantitative PCR) was added into a Vero-cell culture containing two-fold dilutions of test-antibiotic. After a three-week incubation period, cells and supernatant were harvested and growth of *M. genitalium* was determined by quantitative PCR. MIC was expressed as the minimal concentration of the test-antibiotic causing a 99% inhibition of growth when compared to the mean of the control cultures grown without antibiotic. A total of 15 strains from 15 patients were macrolide resistant with MICs >16 mg/L for erythromycin.
Results: The MIC range of solithromycin was ≤0.001-16 mg/L (MIC_{50}: 0.001 mg/L and MIC_{90}: 2 mg/L). For the 25 macrolide susceptible strains, the MICs ranged from ≤0.001 to 0.002 mg/L (MIC_{90}: ≤0.001 mg/L). For the 15 macrolide resistant strains, the MICs ranged from 0.25 to 16 mg/L (MIC_{90}: 4 mg/L). The antimicrobial activity of solithromycin was significantly superior to that of azithromycin, other macrolides, as well as to the other classes of antibiotics under investigation.
Conclusion: Previous studies have shown that solithromycin has a high potency against *Neisseria gonorrhoeae* and *C. trachomatis*, and the present in-vitro observations suggest that solithromycin may show promise as a first-line antibiotic in several STIs. It is tempting to speculate that the high activity of solithromycin would lead to less selection of macrolide resistant *M. genitalium*. Whether the activity will be sufficient also for the majority of macrolide resistant *M. genitalium* strains remains to be determined in clinical studies.

Background

Mycoplasma genitalium is a well-established and important cause of sexually transmitted infections (STIs). The cure-rate after treatment with a 1 g single dose azithromycin (AZM) has been decreasing from nearly 90% in populations without macrolide resistance (Taylor-Robinson & Jensen, 2011) to 40% in the most recent controlled trial (Manhart *et al.*, 2013). High-level macrolide resistance is readily induced in the 5-10% of cases with susceptible strains where eradication fails. Moxifloxacin is currently the only second-line drug, but multi-drug resistant strains of *M. genitalium* are emerging. In this study, we evaluated the *in-vitro* activity of the new fluoroketolide solithromycin, and compared it to other antimicrobials currently used for treatment of *M. genitalium* infections.



EM micrograph showing *M. genitalium* adhering to Vero cells with the specialised tip structure (arrow).

Materials & Methods

***M. genitalium* strains.**

A collection of 40 *M. genitalium* isolates originating from 38 patients were tested. These included the *M. genitalium* G37 type-strain, an early passage of the M30 strain isolated by David Taylor-Robinson in 1980 (Tully *et al.*, 1983), and one isolate kindly provided by Pat Totten, Seattle, USA. The remaining 37 strains were isolated in Copenhagen. Macrolide resistance with mutations in position 2058 or 2059 (*E. coli* numbering) and MIC >16 mg/L for erythromycin was found in 15 strains. The geographical origin of the strains is shown in table 1.

Table 1
Distribution of *M. genitalium* strains according to country of origin and macrolide resistance.

Country of origin	Number of strains	Number of macrolide resistant strains
United Kingdom	2	0
Denmark	7	1
Sweden	12	3
Norway	4	4
France	3	0
Japan	4	0
Australia	7	6
US	1	1

Determination of minimum inhibitory concentration (MIC).

MICs of solithromycin (Cempra Pharmaceuticals, Chapel Hill, NC, USA), azithromycin (Groton Laboratories, Pfizer Inc., Groton, CT, USA), erythromycin (Sigma-Aldrich Denmark, Vallensbaek Strand, Denmark), doxycycline (Sigma-Aldrich Denmark), ciprofloxacin, and moxifloxacin (Bayer Health Care, Lyngby, Denmark) were determined using a cell culture method where a defined inoculum (2500 genome equivalents (geq) by quantitative PCR) was added into a Vero-cell culture containing two-fold dilutions of test-antibiotic (Hamasuna *et al.*, 2005) (Fig. 1). After a three-week incubation period, cells and supernatant were harvested and growth of *M. genitalium* was determined by quantitative PCR. MIC was expressed as the minimal concentration of the test-antibiotic causing a 99% inhibition of growth when compared to the mean of the control cultures grown without antibiotic (Fig. 2).

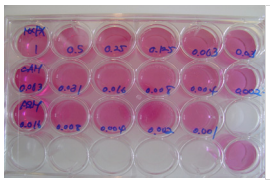


Figure 1. *M. genitalium* growing in Vero cells with various dilutions of antibiotic

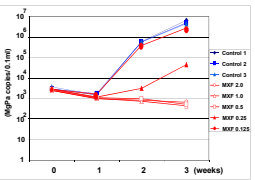


Figure 2. *M. genitalium* growth in Vero cells with various dilutions of antibiotic determined by qPCR

Results

The MIC range of solithromycin was ≤ 0.001 - 16 mg/L (MIC_{50}: 0.001 mg/L and MIC_{90}: 2 mg/L) (Table 2). For the 25 macrolide susceptible strains, the MICs ranged from ≤0.001 to 0.002 mg/L (MIC_{90}: ≤0.001 mg/L) (Figure 3). For the 15 macrolide resistant strains, the MICs ranged from 0.25 to 16 mg/L (MIC_{90}: 4 mg/L). For eight strains with the A2059G mutation, the median MIC was two dilution steps lower than that of the five strains with the A2058G mutation (p=0.02) and lower than that of the two strains with the rare A2058C mutation (p=0.04). The antimicrobial activity of solithromycin was significantly superior to that of azithromycin (p<0.0001) with a median difference of two dilution steps for the macrolide susceptible strains and a median difference of six dilution steps for the macrolide resistant strains (p<0.0001). For erythromycin the difference was even more pronounced with a median difference of 6 dilution steps for the susceptible strains (p<0.0001) (Fig. 3).

The MIC range for doxycycline was 0.06 - 32 mg/L (MIC_{50}: 0.25 mg/L and MIC_{90}: 1 mg/L) and, as would be expected with an antibiotic from a different class , no significant difference between macrolide susceptible and resistant strains was found.

The MIC range for ciprofloxacin was 0.5 - >16 mg/L (MIC_{50}: 2 mg/L and MIC_{90}: 16 mg/L) and for moxifloxacin the range was 0.032 - >16 mg/L (MIC_{50}: 0,125 mg/L and MIC_{90}: 4 mg/L). Interestingly, for five strains from patients failing both azithromycin and moxifloxacin treatment, the MICs for solithromycin were 0.25, 0.5, 0.5, 1, and 4 mg/L, respectively.

Figure 3.
Distribution of SOL, AZI, and ERY MIC for 40 *M. genitalium* strains

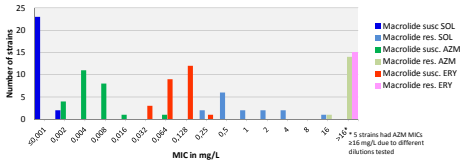


Table 2.
MIC of 35 macrolide susceptible and 15 macrolide resistant strains of *M. genitalium*

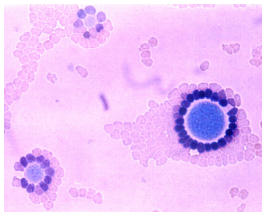
Antibiotic	MIC_{50} mg/L	MIC_{90} mg/L	MIC range mg/L
Solithromycin	0.001	2	≤0.001-16
Azithromycin	0.008	>16	0.002->64
Doxycycline	0.25	1	0.06 - 32
Ciprofloxacin	2	16	0.5 - >16
Moxifloxacin	0.125	4	0.032 - >16

Conclusions

This evaluation showed that the activity of the new, extended-spectrum fluoroketolide solithromycin is superior to that of azithromycin, erythromycin, quinolones and doxycycline. Most importantly, solithromycin was much more active against macrolide resistant *M. genitalium* strains than azithromycin although significant cross-resistance was observed.

Although the number of macrolide resistant strains was limited, the finding that mutations in position 2058 apparently lead to higher solithromycin MICs than those in position 2059 is surprising, as solithromycin has been shown to interact strongly with both residues (Llano-Sotelo *et al.*, 2010).

In Denmark, nearly 40% of all *M. genitalium* strains carry macrolide resistance mediating mutation in the 23S rRNA gene. Of 420 specimens from Denmark carrying resistance mutations, 61% were A2058G and 35% were A2059G. As 4 out of 5 A2058G strains and all A2059G strains had MICs ≤2 mg/l in the present study, this would suggest that approximately 85% of the resistant strains or 94% of all *M. genitalium* would be susceptible to solithromycin. Together with the high potency against *N. gonorrhoeae* and *C. trachomatis*, these *in-vitro* observations suggest that solithromycin may be a promising first-line antibiotic in treatment of STIs such as urethritis and cervicitis. It is tempting to speculate that the high activity of solithromycin would lead to less selection of macrolide-resistant *M. genitalium*.



M. genitalium with adhering human blood cells stained with methylene blue. Hydrogen peroxide production demonstrated by blue colour.

References

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