P 1335
Jørgen Skov Jensen
Statens Serum Institut
Artillerivej 5, DK-2300 Copenhagen S
Denmark
jsj@ssi.dk

Lefamulin is Highly Active *In Vitro* Against Multi-drug Resistant *Mycoplasma genitalium* Strains

JØRGEN SKOV JENSEN [1] AND SUSANNE PAUKNER [2]
[1] Statens Serum Institut, Copenhagen, Denmark, [2] Nabriva Therapeutics AG, Vienna, Austria

STATENS SERUM INSTITUT

Abstract

Background: *Mycoplasma genitalium* is the second most common bacterial sexually transmitted infections (STI) accounting for approximately 25% of non-chlamydial-non-gonococcal urethritis. First-line treatment is azithromycin, but rates of resistance are increasing globally. Moxifloxacin is the only effective second-line therapy but resistance is common, in particular in the Asia-Pacific region and dual resistance to macrolides and fluoroquinolones is making treatment options extremely limited. Thus, alternative therapies for which there is no cross-resistance are urgently needed.

Lefamulin is the first semi-synthetic pleuromutilin antibiotic for IV and oral use in humans. It is currently in Phase 3 trials for the treatment of community-acquired bacterial pneumonia.

Methods: We evaluated the *in vitro* activity of lefamulin against a collection of 41 *M. genitalium* strains including the ATCC G37 type strain, 20 macrolide-susceptible and 20 macrolide resistant clinical isolates. Of the 41 strains, nine were moxifloxacin resistant with eight of these also having combined macrolide resistance, thus characterised as multi-drug-resistant (MDR).

MICs were determined using the Vero cell culture and quantitative real-time PCR method. As comparators, azithromycin, moxifloxacin, and doxycycline were included.

Results: Lefamulin was the most active compound with an MIC_{90} of 0.06 µg/ml compared with azithromycin (MIC_{90} of 16 µg/ml), moxifloxacin (MIC_{90} of 8 µg/ml), and doxycycline (MIC_{90} of 1 µg/ml). Lefamulin remained highly active against both, macrolide- and fluoroquinolone resistant strains.

Conclusions: Lefamulin was highly active against all *M. genitalium* strains tested regardless of their macrolide and fluoroquinolone resistance phenotype. With the growing prevalence of MDR *M. genitalium* strains, further evaluation of lefamulin in a clinical trial is urgently warranted.

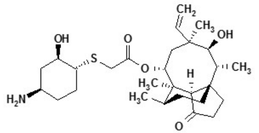

Figure 1: Chemical structure of Lefamulin (BC-3781)

Introduction

Mycoplasma genitalium is an important cause of sexually transmitted infections (STIs) accounting for approximately 25% of non-chlamydial-non-gonococcal urethritis, 15-20% of cervicitis and 10-15% of pelvic inflammatory disease (Taylor-Robinson & Jensen, 2011). It is the second most common bacterial STI, second only to *Chlamydia trachomatis*.

First-line treatment is azithromycin, but rates of resistance are increasing globally and exceeding 40% in most regions. Macrolide resistance is caused by single-base mutations in region V of the 23S rRNA gene, which is present in only one copy in the genome. Thus, one mutational event can change the susceptibility phenotype from extremely susceptible to highly resistant (Jensen & Bradshaw, 2016).

Moxifloxacin is the only effective second-line therapy but mutation rates in the quinolone-resistance-determining-region (QRDR) of ParC as high as 47% have been reported from Japan. A significant proportion of strains have dual resistance to macrolides and fluoroquinolones, making treatment options extremely limited. Thus, alternative therapies for which there is no cross-resistance are urgently needed.

Pleuromutilin antibiotics inhibit bacterial growth by binding to the peptidyl transferase centre of the 50S ribosomal subunit, blocking protein synthesis and have been used to treat mycoplasma infections in swine and poultry for decades. Lefamulin is the first semi-synthetic pleuromutilin antibiotic for IV and oral use in humans (Fig 1). It is currently in Phase 3 trials for the treatment of community-acquired bacterial pneumonia. In the closely related *M. pneumoniae*, lefamulin has been shown to have a very high activity in both macrolide susceptible and resistant strains (Waites *et al*, 2017).

Methods

***M. genitalium* strains.**

A collection of 41 *M. genitalium* isolates originating from 39 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 38 strains were isolated in Copenhagen between 1996 and 2014. Twenty strains were macrolide-resistant, showed azithromycin MICs of ≥16 µg/mL and had mutations in position 2058 (n=9) or 2059 (n=11, *E. coli* numbering). Eight strains had combined resistance to azithromycin and moxifloxacin. 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	Number of multi-drug-resistant strains
UK	2	0	0
Denmark	6	1	0
Sweden	12	4	2
Norway	8	8	4
France	2	0	0
Japan	3	0	0
Australia	7	6	2
USA	1	1	0

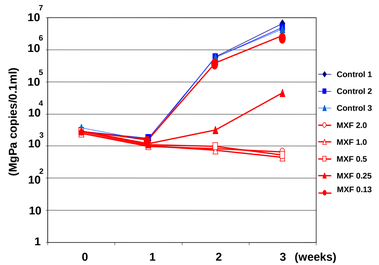

Figure 2. *M. genitalium* growth in Vero cells with various dilutions of antibiotic determined by qPCR

Determination of minimum inhibitory concentration (MIC).

MICs of lefamulin, azithromycin, doxycycline, and moxifloxacin were determined by inoculating 2500 genome equivalents (geq) by quantitative PCR into a Vero-cell culture containing two-fold dilutions of test-antibiotic (Hamasuna *et al.*, 2005). 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).

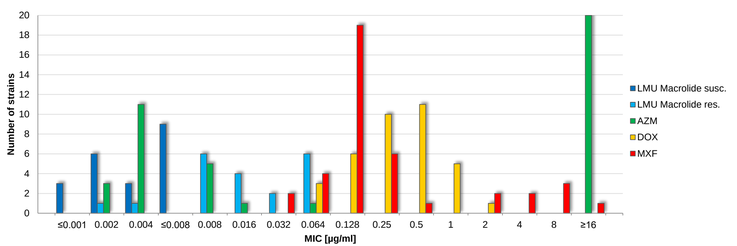

Figure 3: Distribution of LMU, AZI, MXF and DOX MIC for 41 *M. genitalium* strains

Results and Discussion

Lefamulin was the most active compound with an MIC_{90} of 0.06 µg/ml compared with azithromycin (MIC_{90} of >16 µg/ml), moxifloxacin (MIC_{90} of 8 µg/ml), and doxycycline (MIC_{90} of 1 µg/ml) (Table 2). Although the 20 macrolide resistant strains had a significantly higher MIC (MIC_{90} 0.06 µg/ml) than that of the 21 macrolide susceptible strains (MIC_{90} ≤0.008) p<0.0001, lefamulin remained highly active against all macrolide resistant strains (Figure 2).

M. genitalium strains with macrolide resistance mediating mutations in 23S rRNA at position 2058 had lower lefamulin MIC (MIC_{50} 0.008 µg/ml, range 0.002-0.063 µg/ml) than had those with mutations in position 2059 (MIC_{50} 0.03 µg/ml, range 0.008-0.063 µg/ml), p=0.007. The higher lefamulin MICs found in strains with mutations in position 2059 compared to 2058 is different to what is seen for erythromycin against isogenic *M. pneumoniae* mutants (Lucier *et al.*, 1995) and for the ketolide solithromycin against *M. genitalium* (Jensen *et al.*, 2014), where strains with 2058 mutations (some of which were also included in this study) displayed higher macrolide/ketolide MICs. The presence of additional mutations in *rplD* or *rplC* which are known to confer pleuromutilin resistance is still under investigation. Moxifloxacin resistance was equally distributed between the two groups, p=0.6.

MICs for the eight MDR strains was similar to those of the 12 macrolide resistant, moxifloxacin susceptible strains p=0.3 (MIC_{90} 0.063 for both groups). Thus, moxifloxacin resistance did not influence lefamulin MICs.

Table 2.
MICs of 20 macrolide susceptible and 21 macrolide resistant strains of *M. genitalium*

Antibiotic	MIC_{50} [µg/ml]	MIC_{90} [µg/ml]	MIC range [µg/ml]
Lefamulin	≤0.008	0.06	0.0005-0.06
Lefamulin (macrolide suscept.)	0.004	≤0.008	0.0005-≤0.008
Lefamulin (macrolide resistant)	0.016	0.06	0.002-0.06
Azithromycin	0.06	>16	0.002->64
Doxycycline	0.25	1	0.06-2
Moxifloxacin	0.125	8	0.03->16

Conclusions

- Lefamulin was highly active against all *M. genitalium* strains tested, including fluoroquinolone- and macrolide-resistant strains. Despite an increase in the MIC to strains with macrolide resistance mediated via a mutation at position 2058 and 2059 of the 23S rRNA gene, lefamulin MICs remained extremely low.
- With the growing problems of MDR *M. genitalium* strains, particularly in the Asia-Pacific region, a clinical trial of lefamulin for the treatment of *M. genitalium* infections is warranted.
- In addition, work to assess antimicrobial combinations that could potentially protect against the development of resistance to lefamulin, without interfering with its activity is worthy of exploration.

References

Hamasuna R., Y. Osada, J. S. Jensen, *Antimicrob. Agents Chemother.* **49**, 4993 (2005).
Jensen J. S. , C. Bradshaw, *BMC. Infect. Dis.* **15**, 343 (2015).
Jensen J. S. , P. Fernandes, M. Unemo, *Antimicrob. Agents Chemother.* **58**, 3151 (2014).
Lucier T. S. , K. Heitzman, S. K. Liu, P. C. Hu, *Antimicrob. Agents Chemother.* **39**, 2770 (1995).
Taylor-Robinson D., J. S. Jensen, *Clin. Microbiol. Rev.* **24**, 498 (2011).
Tully J. G. , D. Taylor-Robinson, D. L. Rose, R. M. Cole, J. M. Bove, *Int. J. Syst. Bacteriol.* **33**, 387 (1983).
Waites K. B., *D.M. Crabb, L.B. Duffy, J.S. Jensen, Y. Liu, S. Paukner*, *Antimicrob. Agents Chemother.* **61**, e02008 (2017).

27th European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, 22-25 April 2017