AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

SUSCEPTIBILITY

In Vitro Activity of the Novel Pleuromutilin Lefamulin (BC-3781) and Effect of Efflux Pump Inactivation on Multidrug-Resistant and Extensively Drug-Resistant *Neisseria gonorrhoeae*

Susanne Jacobsson,[a] Susanne Paukner,[b] Daniel Golparian,[a] Jörgen S. Jensen,[c] Magnus Unemo[a]

WHO Collaborating Centre for Gonorrhoea and Other Sexually Transmitted Infections, National Reference Laboratory for Pathogenic *Neisseria*, Department of Laboratory Medicine, Faculty of Medicine and Health, Örebro University, Örebro, Sweden[a]; Nabriva Therapeutics AG, Vienna, Austria[b]; Department of Microbiology and Infection Control, Sexually Transmitted Infections, Research and Development, Statens Serum Institut, Copenhagen, Denmark[c]

ABSTRACT We evaluated the activity of the novel semisynthetic pleuromutilin lefamulin, inhibiting protein synthesis and growth, and the effect of efflux pump inactivation on clinical gonococcal isolates and reference strains ($n = 251$), including numerous multidrug-resistant and extensively drug-resistant isolates. Lefamulin showed potent activity against all gonococcal isolates, and no significant cross-resistance to other antimicrobials was identified. Further studies of lefamulin are warranted, including *in vitro* selection and mechanisms of resistance, pharmacokinetics/pharmacodynamics, optimal dosing, and performance in randomized controlled trials.

KEYWORDS gonorrhea, pleuromutilin, treatment, antimicrobial resistance, MtrCDE efflux pump

Received 21 July 2017 **Returned for modification** 18 August 2017 **Accepted** 30 August 2017

Accepted manuscript posted online 11 September 2017

Citation Jacobsson S, Paukner S, Golparian D, Jensen JS, Unemo M. 2017. *In vitro* activity of the novel pleuromutilin lefamulin (BC-3781) and effect of efflux pump inactivation on multidrug-resistant and extensively drug-resistant *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 61:e01497-17. https://doi.org/10.1128/AAC.01497-17.

Address correspondence to Magnus Unemo, magnus.unemo@regionorebrolan.se.

Neisseria gonorrhoeae has developed resistance to all antimicrobials used in monotherapy of gonorrhea (1). Many gonorrhea treatment guidelines currently recommend ceftriaxone 250 to 500 mg × 1 plus azithromycin 1 to 2 g × 1 as empirical first-line treatment (2–6). However, recently the first global treatment failure with recommended dual antimicrobial therapy was verified (7), and new antimicrobials for treatment are crucial (1).

Lefamulin (BC-3781; Nabriva, Vienna, Austria) is a novel semisynthetic pleuromutilin (8, 9; S. Paukner, A. Gruss, T.R. Fritsche, Z. Ivezic-Schoenfeld, and R. N. Jones, poster E-1183, presented at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO, 2013) which inhibits bacterial protein synthesis and growth by binding to the peptidyltransferase center of the 50S ribosomal subunit (interfering with 23S rRNA) with high affinity and specificity and uses different targets than do other antimicrobial classes (9). Lefamulin has completed a phase II clinical randomized controlled trial (RCT) for acute bacterial skin and skin structure infections and is currently undergoing two phase III RCTs for the treatment of community-acquired bacterial pneumonia (10–13).

We evaluated the *in vitro* activity of lefamulin against clinical gonococcal isolates and international reference strains ($n = 251$) and the effect of inactivation of efflux pumps (MtrCDE, MacAB, and NorM). The tested gonococcal isolates were diverse geographically (having essentially global representativeness), temporally (1991 to 2016), and phenotypically/genetically, including 34 international reference strains (all WHO reference strains [14, 15]), 100 consecutive clinical Swedish gonococcal isolates from

TABLE 1 Antimicrobial activities of lefamulin against all *Neisseria gonorrhoeae* isolates and isolate subgroups and susceptibility of all isolates to antimicrobials currently or previously recommended for treatment of gonorrhea

Antimicrobial and isolate group (no.)	MIC (μg/ml)[a]				Susceptibility (%)[b]		
	Range	50%[c]	90%[d]	Modal	S	I	R
Lefamulin							
All isolates (251)	0.004–2	0.25	1	0.5	ND[e]	ND	ND
Contemporary consecutive isolates (100)[f]	0.004–1	0.25	0.5	0.25	ND	ND	ND
Selected resistant isolates (117)	0.016–2	0.5	1	0.5	ND	ND	ND
Azithromycin-resistant isolates (123)	0.016–2	0.5	1	0.5	ND	ND	ND
Azithromycin-susceptible isolates (128)	0.004–1	0.25	0.5	0.5	ND	ND	ND
Reference strains (34)	0.016–2	0.5	1	0.5	ND	ND	ND
Ceftriaxone (251)	<0.002–4	0.008	0.064	0.004	97.2	ND	2.8
Cefixime (251)	<0.016–8	0.016	0.125	<0.016	91.2	ND	8.8
Azithromycin (251)	0.016–>256	0.5	16	16	40.6	10.4	49.0
Spectinomycin (251)	4–1,024	16	16	16	98.0	ND	2.0
Ciprofloxacin (251)	<0.002–>32	0.25	>32	≥32	48.6	0.0	51.4
Ampicillin (251)	0.016–>256	0.5	8	0.125	37.8	47.4	14.7
Tetracycline (251)	0.125–128	2	16	2	25.9	23.1	51.0

[a]MIC was determined using the agar dilution technique for lefamulin and the Etest for the additional antimicrobials. Only whole MIC dilutions are reported.
[b]S, susceptible; I, intermediately susceptible; R, resistant. The EUCAST breakpoints (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.0_Breakpoint_Tables.pdf) were applied for all antimicrobials.
[c]MIC at which 50% of the isolates tested were inhibited.
[d]MIC at which 90% of the isolates tested were inhibited.
[e]Not determined due to lack of interpretative criteria.
[f]Consecutive clinical Swedish gonococcal isolates obtained in 2016.

2016, and 117 isolates selected for their resistance phenotype. The latter included a high proportion (49%) of azithromycin-resistant isolates (macrolides also target the 50S ribosomal subunit [23S rRNA]), extensively drug-resistant (XDR [16]) isolates (7, 17–19), and numerous isolates with *in vitro* or clinical resistance to extended-spectrum cephalosporins (ESCs) and other high-level clinical resistance and multidrug resistance (MDR [16]) to other antimicrobials previously used for gonorrhea treatment.

The MICs of lefamulin were determined by the CLSI agar dilution technique, and MICs of ceftriaxone, cefixime, azithromycin, spectinomycin, ciprofloxacin, ampicillin, and tetracycline were determined by the Etest (AB bioMérieux, Marcy l'Etoile, France). The *mtrD*, *macA*, and *norM* genes, coding for (sub)components of the respective efflux pumps, were inactivated in WHO F, WHO O, WHO P, and WHO X (14), and one clinical strain with high-level azithromycin resistance (HLAziR; azithromycin MIC ≥256 μg/ml), as previously described (20).

Lefamulin showed potent activity against all 251 *N. gonorrhoeae* isolates (Table 1). Briefly, the modal MIC, MIC_{50}, and MIC_{90} values and the MIC range of lefamulin were low, i.e., 0.5 μg/ml, 0.25 μg/ml, 1 μg/ml, and 0.004 to 2 μg/ml, respectively (Table 1).

No significant cross-resistance between lefamulin and the macrolide azithromycin was identified. A total of 123 (49%) azithromycin-resistant isolates, 8 of these with azithromycin MICs of >256 μg/ml, were compared to 128 (51%) azithromycin-sensitive isolates, and the modal MIC, MIC_{50}, MIC_{90}, and MIC range of lefamulin for these groups did not show significant differences (Table 1). The MIC distributions for lefamulin and azithromycin (Fig. 1a) and a comparison of the MIC values also showed a relatively limited correlation (Fig. 1b).

The isolates resistant and susceptible to also the nonmacrolide antimicrobials had similar low lefamulin MICs, and no cross-resistance with lefamulin was identified. For the isolates with ESC resistance and isolates resulting in verified ESC treatment failures (n = 22, 8.8%), including failures caused by "superbugs" such as H041 (18) and F89 (17, 19), the lefamulin MICs were 0.5 μg/ml (n = 16) and 1 μg/ml (n = 6).

Inactivation of the MtrCDE efflux pump in all five strains examined decreased the lefamulin MICs significantly (4- to 6-fold). Inactivation of the MacAB or NorM efflux pump had no significant impact on the lefamulin MICs (Table 2).

The novel pleuromutilin lefamulin had potent *in vitro* activity against a large and

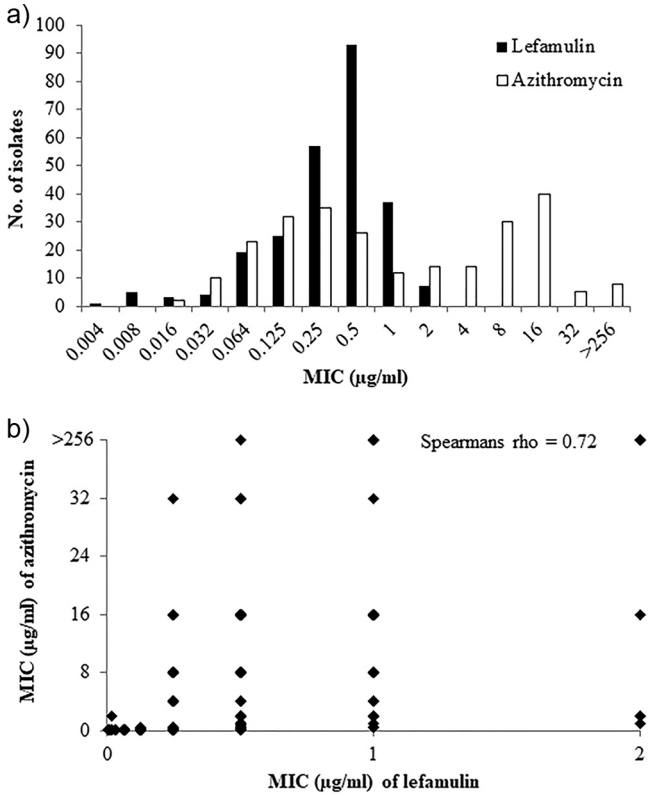

FIG 1 (a) MIC distribution for the novel pleuromutilin lefamulin and the macrolide azithromycin, including clinical *Neisseria gonorrhoeae* isolates (n=217) and international reference strains (*n*=34). (b) Comparison of the MICs of azithromycin and lefamulin for the identical material.

geographically, temporally, and genetically diverse collection of clinical gonococcal isolates and international reference strains, including high-level resistant MDR and XDR isolates. No significant cross-resistance between lefamulin and any other antimicrobials, including the macrolide azithromycin, was identified.

The MtrCDE efflux pump influenced susceptibility to lefamulin, as reported for several antimicrobial classes (20). Further studies on the effect of the MtrCDE efflux pump on lefamulin activity and *in vitro* studies selecting for resistance to lefamulin, using subinhibitory lefamulin concentrations, detailing the resistance determinants in gonococci and investigating the fitness of selected resistant mutants would be valuable to predict the future emergence and transmission of lefamulin resistance.

Lefamulin has finished a successful phase II RCT as a treatment option for acute bacterial skin and skin structure infections and is undergoing two phase III RCTs for treatment of community-acquired bacterial pneumonia (10–13). It has also been shown in phase I clinical trials including healthy subjects that oral and intravenous lefamulin,

TABLE 2 MIC of lefamulin in *Neisseria gonorrhoeae* wild-type strain and strains with the MtrCDE, MacAB, or NorM efflux pump inactivated

Strain	MIC (μg/ml) of:			
	WT[a]	MtrCDE inactivated	MacAB inactivated	NorM inactivated
WHO F	0.125	0.008	0.064	0.064
WHO O	0.5	0.016	0.5	0.5
WHO P	2	0.032	2	2
WHO X	0.5	0.016	0.5	0.5
HLAziR	1	0.064	1	1

[a]WT, wild type.

at exposures predicted to be therapeutically efficacious, is safe and well tolerated, with no significant age, weight, or gender-related differences (W. T. Prince, W. W. Wicha, D. B. Strickman, V. Moschetti, F. Obermayr, and R. Novak, abstr. P906, presented at the 20th European Congress of Clinical Microbiology and Infectious Diseases, 2010; W. W. Wicha, C. Lell, D. K. Logan, and W. T. Prince, abstr. A1-0192010, presented at the 50th Interscience Conference on Antimicrobial Agents and Chemotherapy, 2010). Lefamulin appears to be well tolerated orally and as an injection, with only some local signs and mild, self-limiting symptoms related to the infusion site after the administration of 100 or 150 mg intravenously every 12 h over 5 to 14 days (13). Pharmacokinetic studies have shown rapid penetration of lefamulin into skeletal muscle tissue, subcutaneous adipose tissue, and epithelial lining fluid (21, 22; S. M. Bhavnani, P. G. Ambrose, W. W. Wicha, Z. Ivezic-Schoenfeld, W. T. Prince, C. M. Rubino, abstr. 793, presented at ID Week, 2015). Significant distribution of lefamulin to tissues relevant for sexually transmitted infections (STIs) has also been shown in animals (unpublished data). However, additional pharmacodynamic and pharmacokinetic studies (including evaluation of dosing) and/or modeling, particularly for human urogenital, pharyngeal, and anorectal mucosa, remain imperative to assess lefamulin as a treatment option for gonorrhea. No *in vitro* antagonism of lefamulin in combination with other examined antimicrobials was observed in a study including selected Gram-positive and Gram-negative bacteria (S. Paukner, A. Stoneburner, Z. Ivezic-Schoenfeld, and C. Pillar, abstr E-1161, presented at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO, 2013). Although no STI agents were tested, this is promising for potential future dual antimicrobial therapies for gonorrhea and possibly also for *Mycoplasma genitalium* infections.

In conclusion, lefamulin appears promising for treatment of gonorrhea. However, for lefamulin to become a first-line drug for gonorrhea, lefamulin should have high efficacy (cure >95% of urogenital and extragenital infections), perform well in a combination therapy (to delay resistance development), be widely available and affordable in appropriate quality and dose, have no or minimal drug-drug interactions and low toxicity, be well tolerated, and ideally have activity also against concurrent *Chlamydia trachomatis* and *M. genitalium* infections (S. Paukner, A. Gruss, T.R. Fritsche, Z. Ivezic-Schoenfeld, and R. N. Jones, Poster E-1183, presented at the 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, CO, 2013). Lefamulin fulfills many of these criteria, and further studies are warranted.

ACKNOWLEDGMENTS

We are very grateful to Nabriva Therapeutics AG, Vienna, Austria for providing lefamulin.

The present study was supported by grants from the Örebro County Council Research Committee and the Foundation for Medical Research at Örebro University Hospital, Örebro, Sweden.

This work was performed at the WHO Collaborating Centre for Gonorrhoea and other Sexually Transmitted Infections, National Reference Laboratory for Pathogenic *Neisseria*, Department of Laboratory Medicine, Clinical Microbiology, Örebro University Hospital, Örebro, Sweden.

REFERENCES

1. Unemo M, Shafer WM. 2014. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. Clin Microbiol Rev 27:587–613. https://doi.org/10.1128/CMR.00010-14.
2. WHO. 2016. WHO guidelines for the treatment of *Neisseria gonorrhoeae*. World Health Organization, Geneva, Switzerland.
3. Bignell C, Unemo M, European STI Guidelines Editorial Board. 2013. 2012 European guideline on the diagnosis and treatment of gonorrhoea in adults. Int J STD AIDS 24:85–92. https://doi.org/10.1177/0956462412472837.
4. Workowski KA, Bolan GA, Centers for Disease Control and Prevention. 2015. Sexually transmitted diseases treatment guidelines, 2015. MMWR Recomm Rep 64(RR-03):1–137.
5. Public Health Agency of Canada. 2013. Gonococcal infections. *In* Canadian guidelines on sexually transmitted infections. https://www.canada.ca/en/public-health/services/infectious-diseases/sexual-health-sexually-transmitted-infections/canadian-guidelines/sexually-transmitted-infections/canadian-guidelines-sexually-transmitted-infections-34.html.
6. Australasian Sexual Health Alliance (ASHA). Australian STI management guidelines for use in primary care. www.sti.guidelines.org.au/sexually-transmissible-infections/gonorrhoea#management.
7. Fifer H, Natarajan U, Jones L, Alexander S, Hughes G, Golparian D, Unemo M. 2016. Failure of dual antimicrobial therapy in treatment of gonorrhea. N Engl J Med 374:2504–2506. https://doi.org/10.1056/NEJMc1512757.

8. Kavanagh F, Hervey A, Robbins WJ. 1951. Antibiotic substances from *Basidiomycetes*: VIII. *Pleurotus multilus* (Fr.) Sacc. and *Pleurotus Passeckerianus* Pilat. Proc Natl Acad Sci U S A 37:570–574. https://doi.org/10.1073/pnas.37.9.570.
9. Eyal Z, Matzov D, Krupkin M, Paukner S, Riedl R, Rozenberg H, Zimmerman E, Bashan A, Yonath A. 2016. A novel pleuromutilin antibacterial compound, its binding mode and selectivity mechanism. Sci Rep 6:39004. https://doi.org/10.1038/srep39004.
10. Sader HS, Paukner S, Ivezic-Schoenfeld Z, Biedenbach DJ, Schmitz FJ, Jones RN. 2012. Antimicrobial activity of the novel pleuromutilin antibiotic BC-3781 against organisms responsible for community-acquired respiratory tract infections (CARTIs). J Antimicrob Chemother 67:1170–1175. https://doi.org/10.1093/jac/dks001.
11. Mendes RE, Farrell DJ, Flamm RK, Talbot GH, Ivezic-Schoenfeld Z, Paukner S, Sader HS. 2016. In vitro activity of lefamulin tested against *Streptococcus pneumoniae* with defined serotypes, including multidrug-resistant isolates causing lower respiratory tract infections in the United States. Antimicrob Agents Chemother 60:4407–4411. https://doi.org/10.1128/AAC.00627-16.
12. Paukner S, Sader HS, Ivezic-Schoenfeld Z, Jones RN. 2013. Antimicrobial activity of the pleuromutilin antibiotic BC-3781 against bacterial pathogens isolated in the SENTRY antimicrobial surveillance program in 2010. Antimicrob Agents Chemother 57:4489–4495. https://doi.org/10.1128/AAC.00358-13.
13. Prince WT, Ivezic-Schoenfeld Z, Lell C, Tack KJ, Novak R, Obermayr F, Talbot GH. 2013. Phase II clinical study of BC-3781, a pleuromutilin antibiotic, in treatment of patients with acute bacterial skin and skin structure infections. Antimicrob Agents Chemother 57:2087–2094. https://doi.org/10.1128/AAC.02106-12.
14. Unemo M, Golparian D, Sánchez-Busó L, Grad Y, Jacobsson S, Ohnishi M, Lahra MM, Limnios A, Sikora AE, Wi T, Harris SR. 2016. The novel 2016 WHO *Neisseria gonorrhoeae* reference strains for global quality assurance of laboratory investigations: phenotypic, genetic and reference genome characterization. J Antimicrob Chemother 71:3096–3108. https://doi.org/10.1093/jac/dkw288.
15. Unemo M, Fasth O, Fredlund H, Limnios A, Tapsall J. 2009. Phenotypic and genetic characterization of the 2008 WHO *Neisseria gonorrhoeae* reference strain panel intended for global quality assurance and quality control of gonococcal antimicrobial resistance surveillance for public health purposes. J Antimicrob Chemother 63:1142–1151. https://doi.org/10.1093/jac/dkp098.
16. Tapsall JW, Ndowa F, Lewis DA, Unemo M. 2009. Meeting the public health challenge of multidrug- and extensively drug-resistant *Neisseria gonorrhoeae*. Expert Rev Anti Infect Ther 7:821–834. https://doi.org/10.1586/eri.09.63.
17. Unemo M, Golparian D, Nicholas R, Ohnishi M, Gallay A, Sednaoui P. 2012. High-level cefixime- and ceftriaxone-resistant *N. gonorrhoeae* in France: novel *penA* mosaic allele in a successful international clone causes treatment failure. Antimicrob Agents Chemother 56:1273–1280. https://doi.org/10.1128/AAC.05760-11.
18. Ohnishi M, Golparian D, Shimuta K, Saika T, Hoshina S, Iwasaku K, Nakayama S, Kitawaki J, Unemo M. 2011. Is *Neisseria gonorrhoeae* initiating a future era of untreatable gonorrhea?: detailed characterization of the first strain with high-level resistance to ceftriaxone. Antimicrob Agents Chemother 55:3538–3545. https://doi.org/10.1128/AAC.00325-11.
19. Cámara J, Serra J, Ayats J, Bastida T, Carnicer-Pont D, Andreu A, Ardanuy C. 2012. Molecular characterization of two high-level ceftriaxone-resistant *Neisseria gonorrhoeae* isolates detected in Catalonia, Spain. J Antimicrob Chemother 67:1858–1860. https://doi.org/10.1093/jac/dks162.
20. Golparian D, Shafer WM, Ohnishi M, Unemo M. 2014. Importance of multi-drug efflux pumps in the antimicrobial resistance property of clinical multi-drug resistant isolates of *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 58:3556–3559. https://doi.org/10.1128/AAC.00038-14.
21. Rubino CM, Xue B, Bhavnani SM, Prince WT, Ivezic-Schoenfeld Z, Wicha WW, Ambrose PG. 2015. Population pharmacokinetic analyses for BC-3781 using phase 2 data from patients with acute bacterial skin and skin structure infections. Antimicrob Agents Chemother 59:282–288. https://doi.org/10.1128/AAC.02033-13.
22. Zeitlinger M, Schwameis R, Burian A, Burian B, Matzneller P, Müller M, Wicha WW, Strickmann DB, Prince W. 2016. Simultaneous assessment of the pharmacokinetics of a pleuromutilin, lefamulin, in plasma, soft tissues and pulmonary epithelial lining fluid. J Antimicrob Chemother 71:1022–1026. https://doi.org/10.1093/jac/dkv442.