ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2011, p. 2490–2492 Vol. 55, No. 5
0066-4804/11/$12.00 doi:10.1128/AAC.01623-10

In Vitro Activity of Nifuratel on Vaginal Bacteria: Could It Be a Good Candidate for the Treatment of Bacterial Vaginosis?▽

Giuseppe Togni,[1]* Valeria Battini,[2] Anna Bulgheroni,[3] Federico Mailland,[3] Maurizio Caserini,[3] and Werner Mendling[4]

Microbiology Laboratory, Unilabs SA, Coppet, Switzerland[1]; Microbiology Laboratory, IPAS Institute, Ligornetto, Switzerland[2]; Scientific Department, Polichem SA, Lugano, Switzerland[3]; and Vivantes Clinic for Obstetrics and Gynecology, Berlin, Germany[4]

Received 22 November 2010/Returned for modification 28 December 2010/Accepted 3 February 2011

Bacterial vaginosis is characterized by a shift of the physiological flora to a diverse spectrum of bacteria, where *Gardnerella vaginalis* and *Atopobium vaginae* are the most important markers. In this study, the antimicrobial activity of nifuratel against *G. vaginalis*, *A. vaginae*, and lactobacilli was compared with that of the two currently used antibiotics metronidazole and clindamycin. Results suggest that nifuratel has a better spectrum of activity, being highly active against *G. vaginalis* and *A. vaginae* without affecting lactobacilli.

The microbial flora of the vagina contains high concentrations of a composite population of bacteria (11, 21). It is dominated mainly by lactobacilli that maintain an acidic pH by H_2O_2 and lactic acid production (14). Alterations in this ecosystem can lead to bacterial vaginosis (BV) and *Candida* vaginitis, which account for 90% of vaginal infections (10).

BV is a polymicrobial syndrome characterized by alteration of the vaginal flora, where the normally occurring *Lactobacillus* species are overgrown by endogenous bacteria (24). In particular, high concentrations of *Gardnerella vaginalis* and *Atopobium vaginae* have been shown to be important microbiological markers (1, 18, 27). The association between the presence of *A. vaginae* and BV has been highlighted only recently (8), thanks to its detection by molecular techniques. Although its exact role is not yet fully understood, the association between *A. vaginae* and BV is well established (1, 17, 18, 27), as is its involvement, together with *G. vaginalis*, in the biofilm present on the vaginal epithelium during BV (25).

The therapies of choice for BV are systemic or topical metronidazole and clindamycin. Previous studies reported cure rates of 70 to 96% for both antibiotics, with recurrence rates of 49 to 66%, following 7 days of therapy (2, 13, 16).

Inadequate diagnosis (23), pharmacologic resistance (20), and persistence of an adherent bacterial biofilm after treatment (26) seem to be the main reasons for failures of BV treatment and eradication, as well as the presence of a complex microbial population with putative resistance to antimicrobials.

Nifuratel is a nitrofuran derivative with strong activity against *Trichomonas vaginalis* (4, 7) and a broad spectrum of antibacterial action (7, 19, 22). The purpose of this study was to investigate the potential of nifuratel in the treatment of BV and compare it with metronidazole and clindamycin against *G. vaginalis*, *A. vaginae*, and lactobacilli.

The bacterial strains tested were both clinical isolates and reference strains. Clinical isolates from vaginal swabs (provided by Maditest, Vevey, Switzerland) were grown and identified following standard protocols. In addition, *Lactobacillus* strains were identified by DNA amplification and sequencing (Microsynth, Balgach, Switzerland) (29).

Strains of *A. vaginae* ($n = 10$; CCUG 38953T, CCUG 42099, CCUG 43049, CCUG 44061, CCUG 44116, CCUG 44125, CCUG 44156, CCUG 44258, CCUG 48515, and CCUG 55226; Culture Collection Center, University of Göteborg, Göteborg, Sweden), *G. vaginalis* ($n = 22$; ATCC 14018 and 21 clinical isolates), and *Lactobacillus* spp. ($n = 20$; *Lactobacillus crispatus* CCUG 27076A and 4 clinical strains; *L. iners* CCUG 24626 and 2 clinical strains; *L. gasseri* CCUG 24836; and *L. jensenii* CCUG 35572T and 11 clinical strains) were tested.

Stock solutions of nifuratel (Polichem, Lugano-Pazzallo, Switzerland) and metronidazole (Sigma-Aldrich, Munich, Germany) were prepared in dimethyl sulfoxide (DMSO; Sigma-Aldrich) to a concentration of 51.2 mg/ml. Clindamycin (Sigma-Aldrich) was dissolved in water to a concentration of 0.64 mg/ml. Stock solutions were immediately used or stored at −60°C. Working solutions were obtained by serial 2-fold dilutions in DMSO (nifuratel and metronidazole) or water (clindamycin).

Plates were prepared by following CLSI standard protocols (3). The ranges of concentrations tested were 0.125 to 256 μg/ml for nifuratel and metronidazole and 0.125 to 64 μg/ml for clindamycin.

Inocula were prepared in brucella broth to a 0.5 McFarland standard (1×10^8 to 2×10^8 CFU/ml) by suspending colonies cultured on Columbia blood agar 5% (vol/vol) sheep blood (Labobasi, Novazzano, Switzerland) for 3 days at 36 ± 1°C under anaerobic conditions (*A. vaginae*) or in a CO_2-enriched atmosphere (*G. vaginalis* and *Lactobacillus* spp.).

Brucella agar supplemented with 5 μg hemin, 1 μg vitamin K1 per ml, and 5% (vol/vol) sheep blood (Labobasi) and containing the appropriate antibiotic concentration was inoculated with 2 μl of the bacterial suspension and incubated for 3 days at 36 ± 1°C under anaerobic conditions (*A. vaginae*) or in a CO_2-enriched atmosphere (*G. vaginalis* and *Lactobacillus* spp.) (3).

Quality controls show that supplemented brucella agar medium and the final DMSO concentration in the medium (1%)

* Corresponding author. Mailing address: Unilabs SA, Laboratoire Central de Suisse Romande, Ch. des Perrières 2, C.P. 100, CH-1296 Coppet, Switzerland. Phone: 41 (0)22 716 20 14. Fax: 41 (0)22 716 20 49. E-mail: Giuseppe.togni@unilabs.com.

▽ Published ahead of print on 14 February 2011.

TABLE 1. Frequency distribution of MICs, MIC_{50}, MIC_{90}, and MIC ranges for *G. vaginalis*, *A. vaginae*, and *Lactobacillus* spp.

Organism (total no. of isolates) and antibiotic	No. of isolates with MIC (μg/ml) of:														MIC range	MIC_{50}	MIC_{90}
	<0.125	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256			
A. vaginae (n = 10)																	
NIF[a]		1	2	5	2										0.125–1	0.5	1
MTZ[b]								1	2	3	2	1	1		8–256	32	128
CLI[c]	10														<0.125	<0.125	<0.125
G. vaginalis (n = 22)																	
NIF	3		1	4	3	7	4								<0.125–4	2	4
MTZ	4						1	2	6	5	1	1	1	1	<0.125–>256	16	128
CLI	20			2											<0.125–0.5	<0.125	<0.125
Lactobacillus spp. (n = 20)																	
NIF								2	1		1		2	14	8–>256	>256	>256
MTZ														20	>256	>256	>256
CLI	19				1										<0.125–1	<0.125	<0.125

[a] NIF, nifuratel.
[b] MTZ, metronidazole.
[c] CLI, clindamycin.

did not affect the bacterial growth of all tested strains. Moreover, the MICs of the two control strains, *G. vaginalis* ATCC 14018 and *Bacteroides fragilis* ATCC 25285, were in the acceptable ranges for metronidazole and clindamycin (data not shown).

Our results (Table 1) show that clindamycin is highly active against both *G. vaginalis* (MIC for 90% of the strains tested [MIC_{90}], 0.25 μg/ml) and *A. vaginae* (MIC_{90}, <0.125 μg/ml), in accordance with previous studies on *G. vaginalis* (12, 15) and *A. vaginae* (5). Metronidazole was partially active against *G. vaginalis* (MICs, <0.125 to 256 μg/ml) and *A. vaginae* (MICs, 8 to 256 μg/ml). These results are also in accordance with previously published data (5, 12, 15). Nifuratel was more active on *G. vaginalis* and *A. vaginae* than metronidazole, with MICs ranging from <0.125 to 4 μg/ml and from <0.125 to 1 μg/ml, respectively.

All tested *Lactobacillus* strains were highly susceptible to clindamycin (MICs, 0.125 to 1 μg/ml) and resistant to metronidazole (MICs, >256 μg/ml). Overall, nifuratel was not effective against lactobacilli (MIC_{50}, >256 μg/ml). Only *L. iners* strains (n = 3) appeared to be more sensitive to nifuratel than the other species, with MICs of 8, 16, and 256 μg/ml. It is interesting that previous studies have shown that *L. iners* is more common than other lactobacilli in samples that have a Nugent score of >4 (6) and after metronidazole treatment (9). Moreover, it seems to predispose to some extent to the occurrence of abnormal vaginal microflora (28). Although these observations deal only with three *L. iners* strains, they suggest that nifuratel could not only be useful in the eradication of bacteria associated with BV, like *G. vaginalis* and *A. vaginae*, but also encourage the development of species of *Lactobacillus* other than *L. iners*. Further analysis should be performed to confirm these partial observations.

In conclusion, our results suggest that nifuratel is a good potential candidate for the first-line treatment of BV. Indeed, it is active *in vitro* against the pool of bacteria recognized to cause BV and, conversely, does not affect the normal flora of lactobacilli. Based on these encouraging results, two pivotal clinical studies on oral and topical treatments are ongoing in order to confirm if this antibiotic offers a real advantage over standard BV treatments.

This study was supported by Polichem SA, Lugano, Switzerland.

REFERENCES

1. **Bradshaw, C. S., et al.** 2006. The association of *Atopobium vaginae* and *Gardnerella vaginalis* with bacterial vaginosis and recurrence after oral metronidazole therapy. J. Infect. Dis. **194:**828–836.
2. **Bradshaw, C. S., et al.** 2006. High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. J. Infect. Dis. **193:**1478–1486.
3. **Clinical and Laboratory Standards Institute.** 2007. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard—seventh edition; M11-A7 and M11-S1. Clinical and Laboratory Standards Institute, Wayne PA.
4. **Coppi, F., and V. Bertagnolli.** 1965. Esperienze cliniche in urologia con il metilmercadone nuovo chemioterapico furanico. Urologia **32:**678–683.
5. **De Backer, E., et al.** 2006. Antibiotic susceptibility of *Atopobium vaginae*. BMC Infect. Dis. **6:**51.
6. **De Backer, E., et al.** 2007. Quantitative determination by real-time PCR of four vaginal *Lactobacillus* species, *Gardnerella vaginalis* and *Atopobium vaginae* indicates an inverse relationship between *L. gasseri* and *L. iners*. BMC Microbiol. **7:**115.
7. **Dubini, F., and P. Furneri.** 1985. Attività antimicrobica del nifuratel. G. Ital. Chemioter. **32:**545–552.
8. **Ferris, M. J., et al.** 2004. Association of *Atopobium vaginae*, a recently described metronidazole resistant anaerobe, with bacterial vaginosis. BMC Infect. Dis. **4:**5.
9. **Ferris, M. J., J. Norori, M. Zozaya-Hinchliffe, and D. H. Martin.** 2007. Cultivation-independent analysis of changes in bacterial vaginosis flora following treatment. J. Clin. Microbiol. **45:**1016–1018.
10. **Fleury, F. J.** 1981. Adult vaginitis. Clin. Obstet. Gynecol. **24:**407–438.
11. **Fredricks, D. N., T. L. Fiedler, K. K. Thomas, C. M. Mitchell, and J. M. Marrazzo.** 2009. Changes in vaginal bacterial concentrations with intravaginal metronidazole therapy for bacterial vaginosis as assessed by quantitative PCR. J. Clin. Microbiol. **47:**721–726.
12. **Goldstein, E. J., et al.** 2002. In vitro activities of garenoxacin (BMS 284756) against 108 clinical isolates of *Gardnerella vaginalis*. Antimicrob. Agents Chemother. **46:**3995–3996.
13. **Greaves, W. L., J. Chungafung, B. Morris, A. Haile, and J. L. Townsend.** 1988. Clindamycin versus metronidazole in the treatment of bacterial vaginosis. Obstet. Gynecol. **72:**799–802.
14. **Hawes, S. E., et al.** 1996. Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. J. Infect. Dis. **174:**1058–1063.
15. **Kharsany, A. B., A. A. Hoosen, and J. Van den Ende.** 1993. Antimicrobial susceptibilities of *Gardnerella vaginalis*. Antimicrob. Agents Chemother. **37:**2733–2735.
16. **Koumans, E. H., L. E. Markowitz, and V. Hogan.** 2002. Indications for therapy and treatment recommendations for bacterial vaginosis in nonpregnant and pregnant women: a synthesis of data. Clin. Infect. Dis. **35:**152–172.
17. **Menard, J. P., F. Fenollar, M. Henry, F. Bretelle, and D. Raoult.** 2008.

Molecular quantification of *Gardnerella vaginalis* and *Atopobium vaginae* loads to predict bacterial vaginosis. Clin. Infect. Dis. **47:**33–43.
18. **Menard, J. P., et al.** 2010. High vaginal concentrations of *Atopobium vaginae* and *Gardnerella vaginalis* in women undergoing preterm labor. Obstet. Gynecol. **115:**134–140.
19. **Mendling, W., A. Poli, and P. Magnani.** 2002. Clinical effects of nifuratel in vulvovaginal infections. A meta-analysis of metronidazole-controlled trials. Arzneimittelforschung **52:**725–730.
20. **Nagaraja, P.** 2008. Antibiotic resistance of *Gardnerella vaginalis* in recurrent bacterial vaginosis. Indian J. Med. Microbiol. **26:**155–157.
21. **Oakley, B. B., T. L. Fiedler, J. M. Marrazzo, and D. N. Fredricks.** 2008. Diversity of human vaginal bacterial communities and associations with clinically defined bacterial vaginosis. Appl. Environ. Microbiol. **74:**4898–4909.
22. **Savoia, D., and N. Leoncavallo.** 1970. Investigaciones comparativas sobre algunos compuestos de actividad tricomonicida. Ginecol. Obstet. Mex. **20:** 557–562.
23. **Schwiertz, A., D. Taras, K. Rusch, and V. Rusch.** 2006. Throwing the dice for the diagnosis of vaginal complaints? Ann. Clin. Microbiol. Antimicrob. **5:**4.
24. **Sobel, J. D.** 2005. What's new in bacterial vaginosis and trichomoniasis? Infect. Dis. Clin. North Am. **19:**387–406.
25. **Swidsinski, A., et al.** 2005. Adherent biofilms in bacterial vaginosis. Obstet. Gynecol. **106:**1013–1023.
26. **Swidsinski, A., et al.** 2008. An adherent *Gardnerella vaginalis* biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole. Am. J. Obstet. Gynecol. **198:**97.e1-6.
27. **Verhelst, R., et al.** 2004. Cloning of 16S rRNA genes amplified from normal and disturbed vaginal microflora suggests a strong association between *Atopobium vaginae*, *Gardnerella vaginalis* and bacterial vaginosis. BMC Microbiol. **4:**16.
28. **Verstraelen, H., et al.** 2009. Longitudinal analysis of the vaginal microflora in pregnancy suggests that *L. crispatus* promotes the stability of the normal vaginal microflora and that *L. gasseri* and/or *L. iners* are more conducive to the occurrence of abnormal vaginal microflora. BMC Microbiol. **9:**116–125.
29. **Zucol, F., et al.** 2006. Real-time quantitative broad-range PCR assay for detection of the 16S rRNA gene followed by sequencing for species identification. J. Clin. Microbiol. **44:**2750–2759.