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Can Lactobacillus fermentum LF10 and Lactobacillus acidophilus LA02 in a Slow-release Vaginal Product be Useful for Prevention of Recurrent Vulvovaginal Candidiasis? A Clinical Study

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Objective
To assess the effectiveness of the association of 2 specific strains, Lactobacillus fermentum LF10 (DSM 19187) and Lactobacillus acidophilus LA02 (DSM 21717), specifically formulated in slow-release effervescent tablets, in patients with recurrent vulvovaginal candidiasis.

Study Design
The study was a clinical trial of 58 women diagnosed with recurrent VVC (Z4 culture-confirmed episodes in a 12-mo period). All patients were given 200mg of fluconazole orally as an induction dose for 3 alternate days during the first treatment week. Afterward, the patients were given a new product formulated in slow-release vaginal tablets containing at least 0.4 billion live cells of each of lactobacillus L. fermentum LF10 and L. acidophilus LA02 (first phase of the prophylactic period), on alternate days for 10 consecutive nights. Patients who were still free of symptoms were given 1 vaginal tablet every week for the next 10 weeks (second phase of the prophylactic period). Patients asymptomatic after the total duration of the observation phase (7 mo) were considered as responders.

Results
During the second 10-week prophylactic phase, 49 of 57 (86.0%) patients remained free of clinical recurrence, whereas symptomatic VVC occurred in 8 patients (14.0%). During the 7-month follow-up, 42 patients of 49 (85.7%) were symptom free at the end of the protocol, whereas clinical recurrences occurred in 7 women (14.3%). Overall, 42 of 58 women enrolled in the study (72.4%) experienced no clinical recurrence throughout the 7-month observation phase (responders).

Conclusions
This study strengthens the evidence supporting the use of specific lactobacilli with well-demonstrated activities associated with the creation and maintenance of a vaginal biofilm that hinders the persistence of an infection caused by Candida.

INTRODUCTION

Vulvovaginal candidiasis (VVC) affects between 70% and 75% of adult women during their lifetime, among which approximately 40% to 50% will experience further episodes, and 10% to 15% will develop recurrent vulvovaginal candidiasis (RVVC), which is defined as the occurrence of 3 to 4 episodes of VVC in a period of 12 months.[1] It is predominantly caused by Candida albicans, a commensal dimorphic yeast-like fungal organism that can be found in the normal vaginal environment and gastrointestinal tract.[2]

In most cases of RVVC, no risk factors can be identified, and recurrences are caused by identical C. albicans strains, suggesting C. albicans persistence in the female anogenital area.[3]

Treating RVVC remains challenging: long-term prophylaxis with 150 mg fluconazole once weekly for 6 months results in 91% relapse-free patients at the end of treatment, but symptomatic relapses occur in 57% of patients within 6 months after the cessation of treatment.[4]

Another trial showed that an initial dose of 3_200 mg fluconazole in the first week followed by a decreasing dose maintenance regime in 117 women (without a placebo control group) achieved 90% disease-free patients after 6 months and 77% disease-free patients after 1 year.[5]

A variety of probiotic bacteria have been targeted as potential therapeutic agents.

Possible mechanisms of this protection include inactivation of pathogens by different Lactobacillus products (lactic acid, H2O2, and bacteriocins), competition for epithelial cell attachment sites, and stimulation of the local immune system.[6]

The objective of this study was to assess the effectiveness of a combination of the 2 specific strains L. fermentum LF10 (DSM 19187) and L. acidophilus LA02 (DSM 21717), specifically formulated in slow-release effervescent tablets, to rapidly create an anaerobic vaginal microenvironment after application. The new product was investigated in vivo for its ability to create and maintain a vaginal microenvironment that did not encourage the establishment, propagation or persistence of a candida infection.

PATIENTS AND METHODS

A total of 58 patients with acute symptomatic VVC, confirmed on fresh wet mount microscopy of vaginal fluid, who had a history of RVVC, were enrolled in the study.

The average age of participants was 34.09 years (range, 21 to 47 y). All participants were symptom free during the induction and first phase of the study.

Eligible patients were at least 18 years old, had a symptomatic attack (total severity score Z3) with both pseudohyphae or blastopores on fresh wet mount microscopy of vaginal fluid, and met the criteria for RVVC (Z4 culture-confirmed episodes in a 12-mo period). The severity score was based on the presence of symptoms (eg, pruritus, irritation, and burning) and vulvovaginal signs (eg, erythema, edema, and excoriation, or fissures).[7]

The severity of each sign or symptom was scored on a scale of 0 (absent or normal) to 3 (severe). Exclusion criteria were pregnancy, diabetes mellitus, and treatment with antifungal agents or other products containing lactobacilli in the previous 4 weeks. The level of vulvovaginal discharge was not scored.

All patients who were eligible for inclusion were given 200 mg of fluconazole orally as an induction dose for 3 alternate days during the first treatment week (induction phase).

At the first follow-up visit (on average after 10 d), all patients who were classified as being clinically cured (severity score<3) were eligible for assignment to the prophylactic phase of the study.

In the prophylactic phase, the patients received a new product formulated in slow-release, slightly effervescent vaginal tablets containing at least 0.4 billion live cells of each lactobacillus L. fermentum LF10 and L. acidophilus LA02. In addition, each tablet contained 340 mg of arabinogalactan and 241mg of fructooligosaccharides. The slight effervescence was mediated by the presence of 62 mg of citric acid and 54mg of sodium bicarbonate.

During the first phase of the prophylactic period, 1 vaginal tablet was inserted into the vagina on alternate days for 10 consecutive nights, preferably before going to bed.

Patients who were still free of symptoms and without signs moved on to the second phase of prophylactic therapy, consisting in the use of 1 vaginal tablet every week for the subsequent 10 weeks.

Thereafter, patients were monitored without treatment for 7 months (ie, the observation phase). Patients were required to discontinue the study protocol if they had a clinical recurrence of VVC (score>3).

The treatment was also discontinued if there was an adverse reaction to the prescribed vaginal therapy. The women were divided into 3 groups:

- Responders: patients asymptomatic after the total duration of observation phase (7 mo).
- Partial responders: patients with a clinical relapse during the 7 months of follow-up.
- Nonresponders: patients with clinical recurrence during the prophylactic phase.

The end point was the proportion of women in clinical remission at the end of the observation period (ie, after 7 mo), with cure defined as a clinical severity score of <3. Descriptive statistics are presented as means±SD or as frequencies and associated percentages.

RESULTS

Table 1 and Figure 1 display the results of the protocol treatment and clinical outcomes at follow-up after the end of treatment (see full text on Lippincott Williams & Wilkins Journals).

One patient dropped out during the first phase of the prophylactic period because of reported adverse effects (vaginal burning) and she was considered as nonresponder.

During the second prophylactic phase, 49 of 57 (86.0%) patients receiving the vaginal product containing the 2 lactobacilli remained without a clinical recurrence, whereas symptomatic VVC occurred in 8 patients (14.0%), who were thus considered as nonresponders.

During the 7-month observation period after the cessation of therapy, 42 patients of 49 (85.7%) were symptom free, whereas clinical recurrences occurred in 7 women (14.3%) at different time points, and they were therefore classified as partial responders.

Overall, 42 of 58 women enrolled in the study (72.4%) experienced no clinical recurrence throughout the 7-month observation phase (responders).

DISCUSSION

Uncomplicated VVC, defined as single episodes or with known predisposing factors for occasional relapse, usually responds to treatment, while RVVC cases can be very difficult to treat. Many patients heavily colonised by Candida may remain asymptomatic, whereas others with low or negative colony counts display recurrent symptoms.

The amount of Candida present in the vagina is very important for epithelial cell–mediated signals.

A crucial factor is Candida adhesion with biofilm formation and the subsequent inflammatory response initiated by the interaction of C. albicans with vaginal epithelial cells.[8]

C. albicans can directly affect vaginal epithelial cells by skewing their cytokine production to a predominantly proinflammatory response.[9] Furthermore, the release of cytokines (chemokines) by vaginal epithelial cells after contact with C. albicans is a critical mechanism for the induction of vaginitis, as chemokines recruit inflammatory cells from the environment, thereby enhancing the release of inflammatory mediators.

Biofilms are communities of microorganisms that are embedded in an extracellular matrix forming a complex 3-dimensional architecture, and it is estimated that 80% of human infections result from pathogenic biofilms.

The capacity of vaginal yeasts to form biofilms seems to be an important attribute influencing the occurrence of RVVC by C. albicans and possibly nonalbicans species, as it can make the elimination of the vaginal yeasts more difficult.[10]

Novel therapeutic or preventive measures that include the use of probiotics have been suggested for 3 decades.

Probiotics are well known for their ability to lower the intravaginal pH, thus establishing a barrier effect against many types of yeasts. Some strains are also able to exert additional and more focused antagonistic activities mediated by specific molecules such as hydrogen peroxide and bacteriocins.[11] This study suggests that L. fermentum LF10 and L. acidophilus LA02 are effective in the treatment of RVVC, and, in fact, about 72% of patients experienced no clinical recurrence throughout the 7-month follow-up period.

Between the end of therapy and the last observation point, after 7 months, only 7 (14.3%) women experienced a relapse. Compared with other published RVVC regimens, our combined treatment showed encouraging results: 7 months after cessation of treatment, a recurrence rate of 14.3% was observed, whereas other studies reported recurrence rates equal to 45% and 57%.4,5

Our long-term goal is to find a lactobacillus strain, or an association of strains, for vaginal application to prevent the recurrence of vulvovaginal infections. One of the main steps in the formulation of the product requires the intensive search for autochthonous strains with adjunctive characteristics that include the capability to form biofilm.

Biofilms could facilitate and promote vaginal colonization and persistence of beneficial strains in the organ, thus allowing the expression of the protective features.

Previous in vitro studies concerning the probiotic properties of L. fermentum showed this species to be an excellent candidate for adhesion to the vaginal epithelium. The species presented intermediate hydrophobicity and an ability to self-aggregate and produce a biofilm on the surface of epithelial cells.[12] It was also found that L. acidophilus could reduce vaginal colonization and infection by Candida in women with RVVC.[13]

We used an active product formulated in slow-release, slightly effervescent vaginal tablets containing live cells of each lactobacillus L. fermentum LF10 and L. acidophilus LA02.

In addition, each tablet contained 340mg of arabinogalactan and 241mg of fructooligosaccharides, 2 fibers used to enhance vaginal colonization by the 2 lactobacilli.

The slight effervescence was mediated by the presence of 62 mg of citric acid and 54 mg of sodium bicarbonate and was intended in particular to rapidly create an anaerobic vaginal microenvironment able to favor the growth of lactobacilli and, in contrast, the formation of biofilms.

In a previous study, the same product was able to significantly resolve Candida yeast symptoms after 28 days in 26 patients of 30 (86.6%): at the end of the second month, recurrences were recorded, although not particularly serious, in only 3 of 26 patients.[14]

The patients applied a vaginal tablet once a day for 7 consecutive nights, followed by 1 tablet every 3 nights for a further 2-week application (acute phase) and, finally, 1 tablet per week for 1 month.

McMillan and colleagues performed in vitro experiments to examine the structure of biofilms associated with aerobic vaginosis, urinary tract infections, and bacterial vaginosis. Uropathogenic Escherichia coli were able to form relatively thin biofilms within 5 days (6 mm height), whereas Atopobium vaginae and Gardnerella vaginalis formed thicker biofilms (12 mm in height) within 2 days.[15]

Therefore, it is crucial to interfere with biofilm formation early after the drastic reduction of the Candida concentration (induction phase with fluconazole), and then to maintain the “positive” biofilm with a greater interval of use of the lactobacilli.

In fact, in our study we used a protocol where the prophylactic period was conducted with an intensive first phase (10 d) intended to allow the creation of a protective biofilm, followed by a second phase of stabilization with the use of 1 vaginal tablet every week for the subsequent 10 weeks.

We observed a higher incidence of relapses during the treatment compared with the follow-up period, thus suggesting the tendency of the protective biofilm to self-renew after initial establishment.

Only 1 drop-out was recorded, thus confirming the very high tolerability profile of the product. This is probably related to the decision to use the product on alternate days in the first phase of prophylaxis. In fact, the slight effervescence of the tablets could create minor irritative phenomena combined with the secretion of specific vaginal cytokines.

Finally, it is worth mentioning that the previous pilot trial using the same slow-release product did not examine the ability of lactobacilli to prevent recurrences in women who already had RVVC.

However, a placebo-controlled trial with a larger population should be carried out to clarify whether this new slow-release product can be used effectively for the prophylaxis of recurrent episodes of VVC.

In any case, this study strengthens the evidence supporting the use of specific beneficial strains with welldemonstrated activity associated with the creation and maintenance of a vaginal biofilm that hinders the persistence of an infection caused by Candida.

REFERENCES

1. Sobel JD. Vulvovaginal candidosis. Lancet. 2007;369: 1961–1971.

2. Cetin M, Ocak S, Gungoren A, et al. Distribution of Candida species in women with vulvovaginal symptoms and their association with different ages and contraceptive methods. Scand J Infect Dis. 2007;7:584–588.

3. Vazquez JA, Sobel JD, Demitriou R, et al. Karyotyping of Candida albicans isolates obtained longitudinally in women with recurrent vulvovaginal candidiasis. J Infect Dis. 1994;170: 1566–1569.

4. Sobel JD, Wiesenfeld HC, Martens M. Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. N Engl J Med. 2004;351:876–883.

5. Donders G, Bellen G, Byttebier G, et al. Individualized decreasing-dose maintenance fluconazole regimen for recurrent vulvovaginal candidiasis (ReCiDiF trial). Am J Obstet Gynecol. 2008;199:613.e1–613.e9.

6. Aroutcheva A, Gariti D, Simon M, et al. Defense factors of vaginal lactobacilli. Am J Obstet Gynecol. 2001;185:375–379.

7. Sobel JD, Kapernick PS, Zervos M, et al. Treatment of complicated Candida vaginitis: comparison of single and sequential doses of fluconazole. Am J Obstet Gynecol. 2001;185:363–369.

8. Barousse MM, Espinosa T, Dunlap K, et al. Vaginal epithelial cell anti-Candida albicans activity is associated with protection against symptomatic vaginal candidiasis. Infect Immun. 2005;73:7765–7767.

9. Steele C, Fidel PL Jr. Cytokine and chemokine production by human oral and vaginal epithelial cells in response to Candida albicans. Infect Immun. 2002;70:577–583.

10. Chassot F, Negri MFN, Svidzinski AE, et al. Can intrauterine contraceptive devices be a Candida albicans reservoir? Contraception. 2008;77:355–359.

11. Martinez R, Seney S, Summers K, et al. Effect of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 on the ability of Candida albicans to infect cells and induce inflammation. Microbiol Immunol. 2009;53:487–495.

12. Pascual LM, Daniele MB, Ruiz F, et al. Lactobacillus rhamnosus L60, a potential probiotic isolated from the human vagina. J Gen Appl Microbiol. 2008;54:141–148.

13. Falagas ME, Betsi GI, Athanasiou S. Probiotics for prevention of recurrent vulvovaginal candidiasis: a review. J Antimicrob Chemother. 2006;58:266–272.

14. Vicariotto F, Del Piano M, Mogna L, et al. Effectiveness of the association of 2 probiotic strains formulated in a slow release vaginal product, in women affected by vulvovaginal candidiasis: a pilot study. J Clin Gastroenterol. 2012;46:S73–S80.

15. McMillan A, Dell M, Zellar MP, et al. Disruption of urogenital biofilms by lactobacilli. Colloids Surf B Biointerfaces. 2011;86:58–64.