

Oral probiotics can resolve urogenital infections

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Abstract

We report the first clinical evidence that probiotic lactobacilli can be delivered to the vagina following oral intake. In 10 women with a history of recurrent yeast vaginitis, bacterial vaginosis (BV) and urinary tract infections, strains *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 suspended in skim milk and given twice daily for 14 days, were recovered from the vagina and identified by morphology and molecular typing within 1 week of commencement of therapy. In six cases of asymptomatic BV or intermediate BV (based upon Nugent scoring) was resolved within 1 week of therapy. © 2001 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Urogenital infections, including urinary tract infections (UTI), bacterial vaginosis (BV) and yeast vaginitis, afflict an estimated 1 000 000 000 people in the world annually. For patients suffering from BV, they have an increased risk of preterm labor and acquisition of sexually transmitted disease [1]. While antimicrobial agents are quite effective at providing clinical cure for bacterial infections, urogenital pathogen drug resistance is on the increase: those for *Escherichia coli* are as high as 18% in the US and Canada for trimethoprim/sulfamethoxazole [2,3] and 30% in Spain for fluoroquinolones [4]. These findings make it imperative that alternative strategies to disease management be developed.

One potential approach is the use of probiotics (essen-

tially viable bacteria which confer a health benefit to the host). A number of reliable [5] and unreliable [6] probiotic products are used extensively around the world, and indeed one *Lactobacillus casei* Shirota strain, present in Yakult drink (Yakult, Tokyo, Japan) is said to be taken by 24ψ000 000 people each day. However, none of the reliable products have been designed for urogenital application, and their strain contents have no reported effectiveness against urogenital pathogens.

Studies in our laboratory, carried out over the past 20 years, have shown that specially selected probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* B-54 or RC-14, inserted into the vagina can colonize and compete against uropathogens and reduce the risk of UTI [7,8]. If these lactobacilli were able to be delivered to the vagina by oral ingestion, it would represent a major advance, for example in pregnant women with BV. In addition, oral use could provide a practical way for women, many of whom are in developing countries, at high risk of sexually transmitted diseases to potentially better manage their urogenital health. Preliminary data in three volunteers showed that *L. rhamnosus* GR-1 and *L. fermentum* RC-14 colonized the intestine, as proven by recovery in stool 1 week after therapy. Therefore, the present study was set up to determine whether oral intake of GR-1 and RC-14 could be used to deliver the organisms to the vagina by natural colonization and ascension.

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2. Materials and methods

2.1. Probiotic bacteria

Probiotic organisms, *L. rhamnosus* GR-1 and *L. fermentum* RC-14, were selected on the basis of their production of various antagonistic factors against urogenital pathogens [5,9,10] including biosurfactants which inhibit adhesion of Gram-positive cocci including enterococci, staphylococci and Group B streptococci, and Gram-negative rods including coliforms and *Gardnerella*. The organisms were grown in MRS broth in 5% CO₂ at 37°C, harvested and washed in phosphate-buffered saline and resuspended in sterilized skim milk. A probiotic solution containing > 10⁹ viable *L. rhamnosus* GR-1 and *L. fermentum* RC-14 suspended in 3 ml sterilized skim milk (stored at –20°C) was then administered to patients.

2.2. Patients and methods of delivery and recovery of the lactobacilli

Each morning and last thing at night for 14 days, ten women, asymptomatic for infection but with a recent history of urogenital infection, swallowed 3 ml probiotic suspension. The patients provided urine and vagina swabs pre-treatment and 1, 2, 3, and 4 weeks (and in 3 cases weeks 8 and 12) after commencement of the therapy. The swabs were suspended in 1 ml saline and cultured on MRS agar. Morphotypes of lactobacilli, particularly those suspected of being strains *L. rhamnosus* GR-1 and

L. fermentum RC-14 were further subcultured, Gram-stained and ribotyped. The ribotyping consisted of restriction of the genome with endonucleases, separation of the DNA by agarose gel electrophoresis and hybridization with rRNA (16S and 23S) from *E. coli* as described in full previously [11]. The fingerprints characteristic of *L. rhamnosus* GR-1 and *L. fermentum* RC-14 were identified. Controls consisted of a range of ATCC type strains of *Lactobacillus*, markers and samples without lactobacilli. Each patient was asked to record any side effects such as bladder or vaginal irritation, discharge, or intestinal upset, and to inform us of any use of antimicrobial agents for infections occurring during the study.

3. Results

The results showed that *L. rhamnosus* GR-1 and/or *L. fermentum* RC-14 were recovered from the vagina within one week in all 10 patients (Table 1). Patient AL did not provide samples after week 1 and patient SH received antibiotic therapy for bronchitis after week 3. In three of the patients who provided vaginal samples at week 8 and 12, *L. rhamnosus* GR-1 and *L. fermentum* RC-14 were recovered. No side effects were noted. All patients reported improved well being with therapy. This included relief of symptoms of urogenital infection, and reportedly no need for monthly yeast therapy. In the case of JA, the enterococci (present as 1000 per ml urine prior to therapy) were eradicated from her bladder and vagina (from

Table 1
Presence of lactobacilli and identification of *L. rhamnosus* GR-1 and *L. fermentum* RC-14

Patient	One year history	Preswab	Week of swab collection post start of therapy on day 1					
			1	2	3	4	8	12
CK	YV	no lacto	++ GR-1	++ GR-1	++ GR-1	++ GR-1,RC-14	++ GR-1	++ GR-1
TR	YV, UTI	low lacto	+ GR-1	++ GR-1	NS	++ RC-14	++ GR-1	++ GR-1,RC-14
SH	YV	no lacto	+ GR-1	+++ GR-1,RC-14	++ RC-14	++ Ant RC-14		
BC	BV	low lacto	+ RC-14	++ GR-1	++ RC-14	+ RC-14		
AD	YV	lacto	+ GR-1	+ GR-1	+ GR-1	++ GR-1		
AC	YV	lacto	+ RC-14	+ RC-14	NS	++ RC-14		
SB	BV,YV	low lacto	+ RC-14	+ RC-14	+ RC-14			
SO	YV	lacto	++ GR-1	++ GR-1	++ GR-1,RC-14	++ GR-1,RC-14		
JA	UTI,YV	low lacto	++ GR-1 and RC-14 both recovered at each sampling time	++ NS	++ NS	++ NS	++	++
AL	UTI,YV	lacto	+ RC-14	NS	NS	NS		

YV = recurrent yeast vaginitis; BV = recurrent bacterial vaginosis; UTI = recurrent urinary tract infections in past year; no lacto = MRS agar plate culture isolated no lactobacilli; low lacto = less than 10 colonies at zero dilution; +, ++, +++ = one, two, or three lactobacilli isolated by colony morphology and Gram stain; GR-1, RC-14 = identification of *L. rhamnosus* GR-1 or *L. fermentum* RC-14 by colony and Gram stain morphology, and molecular typing; Ant = patient prescribed antibiotics for bronchitis; NS = no sample collected

200 000 to 0 per ml) within 7 days. At 16 months follow-up and continuing daily intake of *L. rhamnosus* GR-1 and *L. fermentum* RC-14, patient JA has remained infection-free.

Nine of the 10 patients had a history of recurrent yeast vaginitis, two of recurrent BV, and three of recurrent UTI, but no such infections arose during treatment and follow-up. Based upon the Nugent scoring system [12] which classifies the vaginal flora as BV, intermediate or normal, six patients (CK,TR,SH,BC,SB,JA) had an abnormal to BV flora which resolved with 1 week of probiotic treatment.

4. Discussion

This study is significant for a number of reasons. Unlike antibiotic therapy used to prevent recurrence of UTI, there were no side effects and patients reported improved well-being and relief from their monthly cyclical recurrences of yeast vaginitis, BV or UTI. Although no clinical efficacy can be claimed in the six cases where the vaginal flora indicated BV or intermediate at the beginning of the study and was restored to normal (dominated by lactobacilli) on therapy, the results make a good case for a larger, randomized, placebo-controlled trial.

The critical finding was the first conclusive proof that two probiotic lactobacilli, specifically selected for their ability to inhibit urogenital pathogen growth and adhesion, can colonize the vagina following oral intake. Notably, in each patient, one or both of the strains colonized the vagina, and remained several months thereafter. This means that the organisms successfully survived the low pH and bile salt of the stomach, and passage through the intestine, and that they then ascended without functional intervention, into the vagina. This now makes it possible to use oral delivery to convey health benefits to the urogenital tract. Both strains are acid and bile tolerant, but it is not clear what mechanisms they used to colonize the intestine and vagina. Probiotic therapy has been shown to be effective against diarrheal diseases [13,14], the world's second most common cause of death, and to confer a range of other health benefits [15]. While *L. rhamnosus* GR-1 and *L. fermentum* RC-14 have not been investigated fully to act in the intestine, their ability to colonize and inhibit the growth and adhesion of intestinal pathogens (unpublished data) could make them useful for this purpose, as well as for the urogenital tract. As other intestinal strains lack some of the properties believed to be important for vaginal colonization [16,17], the use of GR-1 and RC-14 in combination could have clinical importance.

The ability of *L. rhamnosus* GR-1 and *L. fermentum* RC-14 to colonize the vagina confirms earlier data [7,8]. The finding that GR-1 colonized particularly well in some patients, and RC-14 in others stresses the importance of

using more than one organism in probiotic products. Strain *L. fermentum* RC-14 produces hydrogen peroxide, a property believed to be important in the healthy flora of the vagina [18], while *L. rhamnosus* GR-1 resists the killing action of spermicide nonoxynol-9. Thus, the two strains provide combined advantages to colonize the vagina and potentially restore the urogenital flora in women with a history of BV, yeast vaginitis or UTIs.

Although only a limited set of strains have any proven clinical effect or scientific basis [5], there are sufficient data to suggest that this approach could provide a valuable alternative to antibiotic prophylaxis and treatment of infection. By delivering properly selected lactobacilli strains orally, it is hereby shown that the healthy flora of the vagina can also be restored, thus potentially providing a practical means to reduce the risk of vaginal and bladder infections. In developed as well as developing countries, such probiotic products could help relieve pain and complications associated with infection [19], and also provide a vehicle to deliver other nutritional benefits.

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