ELSEVIER

International Journal of Antimicrobial Agents 28S (2006) S30–S34

INTERNATIONAL JOURNAL OF Antimicrobial Agents

www.ischemo.org

A pilot study evaluating the safety and effectiveness of *Lactobacillus* vaginal suppositories in patients with recurrent urinary tract infection

Shinya Uehara [a,*], Koichi Monden [a], Koji Nomoto [b], Yuko Seno [a], Reiko Kariyama [a], Hiromi Kumon [a]

[a] *Department of Urology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata, Okayama 700-8558, Japan*

[b] *Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi, Tokyo 186-8650, Japan*

Abstract

Changes in the indigenous vaginal microflora with uropathogenic bacteria can predispose women to frequently recurring bacterial cystitis. Lactobacilli used as probiotics have played an important role in preventing the colonization of pathogenic bacteria in the vagina. A prospective clinical pilot study was performed to confirm the safety and effectiveness of *Lactobacillus* vaginal suppositories against the recurrence of bacterial urinary tract infection (UTI). The patients enrolled in the study were instructed to administer vaginal suppositories containing the strain *Lactobacillus crispatus* GAI 98322. A significant reduction in the number of recurrences was noted, without any adverse complication ($P = 0.0007$). The administration of vaginal suppositories containing *L. crispatus* GAI 98332 seemed to be a safe and promising treatment for the prevention of recurrent UTI.

Keywords: Probiotics; *Lactobacillus*; Urinary tract infections; Vaginal suppositories

1. Introduction

Although most cases of urinary tract infection (UTI) can be treated easily with recently developed broad-spectrum antibiotics, these antibiotics do not change a patient's susceptibility to recurrences. We have frequently observed cystitis-prone women, even when urological examinations show no detectable underlying conditions of the urinary tract. The patient's quality of life is affected and many women become frustrated by the repeated use of antimicrobial agents whose effectiveness is diminishing owing to their increasing antimicrobial resistance [1]. Therefore, a new strategy for the prophylaxis of recurrent UTI in women should be developed.

There are five strategies that are either presently advocated or under investigation for the prevention of UTI: (i) antibiotics, including natural peptides; (ii) functional foods, including cranberry juice; (iii) vaccines; (iv) probiotics; and (v) miscellaneous, including the avoidance of spermicides and maintaining good hygiene [2]. Long-term antibiotic prophylaxis is the most common method for managing recurrent UTI. However, antibiotic use leads to the increased presence of drug-resistant organisms and many patients suffer from yeast vaginitis as a result of the disruption of normal levels of intestinal and vaginal flora. In such cases, it has been noted that probiotics, which are defined as living microorganisms that can be administered to promote the health of the host [3] by treating or preventing disease, can be used as an alternative preventative approach. The flora of the urogenital tract is abnormal in patients with recurrent UTI compared with those of healthy women [4–6]. This fact leads to the investigation of the role of the flora, particularly lactobacilli, in maintaining urogenital health and reducing the risk of infections. The use of probiotics to restore the normal vaginal flora and to provide a competitive bacterial barrier is becoming increasingly acceptable.

Many publications describing the relationships between lactobacilli and UTI are available [2,7–9]. However, the effectiveness of *Lactobacillus* vaginal suppositories on recurrent UTI is still inconclusive [10,11].

* Corresponding author. Tel.: +81 86 235 7287; fax: +81 86 231 3986.
E-mail address: uesinn@md.okayama-u.ac.jp (S. Uehara).

0924-8579/$ – see front matter © 2006 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.
doi:10.1016/j.ijantimicag.2006.05.008

In this pilot study, we examined the safety and effectiveness of vaginal suppositories by using a hydrogen peroxide-producing *Lactobacillus crispatus* strain in women experiencing recurrent UTI.

2. Materials and methods

2.1. Bacterial strains

Lactobacillus crispatus was selected because it is readily isolated from the vagina of healthy women. Three strains (GAI 98332, GAI 99098, GAI 99099) were kindly provided by K. Watanabe (Division of Anaerobe Research, Life Science Research Center, Gifu University, Japan).

2.2. Hydrogen peroxide production assay

For selection of the most suitable strain, hydrogen peroxide production, an intrinsic protective mechanism in the vaginal compartment, was measured for *L. crispatus* strains (GAI 98332, GAI 99098 and GAI 99099). Bioxytech® H_2O_2-560 (Oxis International Inc., Portland, OR, USA) was used for the quantitative hydrogen peroxide assay. Three strains were cultured in de Man–Rogosa–Sharpe (MRS) broth (Becton Dickinson and Co., Franklin Lakes, NJ, USA) for 24 h at 37 °C. From the precultured medium, 50 μL was added to modified Rogosa broth (1%, w/v Trypticase peptone (Becton Dickinson); 0.5%, w/v yeast extract (Becton Dickinson); 0.3%, w/v tryptose (Becton Dickinson); 0.8%, w/v KH_2PO_4 (Wako Chemical Co., Osaka, Japan); 0.298%, w/v K_2HPO_4 (Wako); 0.5%, w/v glucose (Wako); 0.2%, w/v diammonium hydrogen citrate (Wako); 0.1%, w/v Tween 80 (Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan); pH 6.8) and the culture was incubated at 37 °C. After 36 h of incubation, 10 μL of the culture was used for the hydrogen peroxide production assay. The assays were repeated five times and the values were presented as the mean ± standard deviation (S.D.).

2.3. Viability of L. crispatus GAI 98332 in vaginal suppositories

Lactobacillus vaginal suppositories containing *L. crispatus* GAI 98332, 1.0×10^8 CFU (colony-forming units) per one suppository, were made at our institute. Freeze-dried bacterium was mixed and solidified with Witepsol H15 (Warner Graham Co., Cockeysville, MD, USA). The viability of *L. crispatus* GAI 98332 in vaginal suppositories was investigated to confirm the stability of those stored at 4 °C. After 1, 2, 4 and 8 weeks, five suppositories for each time point were melted in five sterile tubes containing 10 mL of saline for 10 min at 37 °C, and the tubes then vortexed. After a serial 10-fold dilution of the samples in saline, a volume of 100 μL of each diluted sample was added to sterile dishes containing 15 mL of MRS agar (Becton Dickinson), and the dishes carefully mixed. Each dish was overlaid with 3 mL of MRS agar after the agar had solidified, and was then incubated at 37 °C under 5% CO_2. After 48 h of incubation, the number of CFU per dish was counted.

2.4. Subjects

Nine female patients who had experienced more than two episodes of UTI in the preceding 12 months and were suffering from recurrent UTI for at least 2 years were included in the pilot study. Seven patients had no detectable underlying conditions of the urinary tract, whereas two patients had neurogenic bladder disturbances and performed clean intermittent catheterization. Each patient read, understood and signed a consent form approved by the Human Ethics Review Board of the Okayama University. The patients were instructed to insert a vaginal suppository containing *L. crispatus* GAI 98332 every 2 days for 1 year before going to bed. This regimen was chosen because we have not yet obtained any convincing data on vaginal colonization with *L. crispatus* GAI 98332. The patients visited our hospital every month and subjective symptoms were examined. Urinalysis and culture of urine and the vagina were performed. An antimicrobial agent was given to any patient with clinical signs and symptoms of UTI, and the study's protocol was recommenced after the UTI was cured. The incidence of UTI before and during treatment with vaginal suppositories containing *L. crispatus* GAI 98332 was compared.

2.5. Statistical analysis

The data were analysed using Mann–Whitney's exact test.

3. Results

3.1. Production of hydrogen peroxide by L. crispatus strains

The highest level of hydrogen peroxide was produced by the strain *L. crispatus* GAI 98332, as shown in Table 1. Thus, this strain was chosen for the study's *Lactobacillus* vaginal suppositories.

3.2. Viability of L. crispatus GAI 98332 in vaginal suppositories

The number of CFU did not decrease over time, as shown in Fig. 1. Thus, the stability of vaginal supposito-

Table 1
Production of hydrogen peroxide by *Lactobacillus crispatus* strains

Strain	Hydrogen peroxide (mM)[a]
GAI 98332	32.3 ± 4.1
GAI 99098	7.3 ± 0.5
GAI 99099	24.5 ± 0.1

[a] The assays were repeated five times for each strain.

Table 2
Case summary before treatment of patients with vaginal suppositories containing *Lactobacillus crispatus* GAI 98332

Patient	Age (years)	Duration of suffering (years)	Total number of recurrent UTIs over 1 year	Causative organisms	Clean intermittent catheterization
1	70	4	4	*E. cloacae*, *E. coli*	Yes
2	78	3	4	*E. faecalis*, *E. coli*	No
3	37	16	6	*E. faecalis*	No
4	39	2	6	*E. faecalis*	No
5	37	15	8	*E. coli*	No
6	55	8	6	*E. coli*	No
7	53	8	4	*E. faecalis*	Yes
8	80	4	4	*P. mirabilis*	No
9	66	4	3	*E. coli*	No

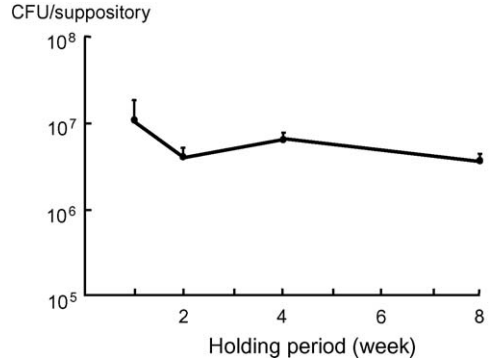

Fig. 1. The viability of *Lactobacillus crispatus* GAI 98322 in vaginal suppositories. The mean value ($N = 5$) for the number of CFU/suppository is plotted (mean ± standard deviation).

ries containing *L. crispatus* GAI 98332 at 4 °C was confirmed.

3.3. Clinical outcome of patients

Nine patients experiencing recurrent UTI participated in this pilot study. Two of nine patients performed clean intermittent catheterization when necessary and seven patients had uncomplicated UTI. Participants' mean age was 57.2 ± 17.2 years, and had suffered from recurrent UTI for 7.1 ± 5.2 years. The duration of treatment with vaginal suppositories containing *L. crispatus* GAI 98332 was 12 months.

Table 3
Case summary during treatment of patients with vaginal suppositories containing *Lactobacillus crispatus* GAI 98332

Patient	Duration of treatment (months)	Total number of recurrent UTIs over 1 year	Causative organisms
1	12	2	*E. coli*
2	12	1	*S. epidermidis*
3	12	0	Not applicable
4	12	1	*E. faecalis*
5	12	4	*E. coli*
6	12	1	*E. coli*
7	12	2	*E. coli*
8	12	0	Not applicable
9	12	1	*E. faecalis*

UTIs, urinary tract infections.

The patients did not report any side effects associated with the study treatment. The culture findings showed that *Escherichia coli* was isolated from urine most frequently before and during treatment (Tables 2 and 3). A significant reduction was observed in the number of recurrences during treatment (from an average of 5.0 ± 1.6 episodes per year to 1.3 ± 1.2, $P = 0.0007$).

4. Discussion

This study demonstrated that vaginal suppositories with *L. crispatus* GAI 98332 can reduce the recurrence of UTI significantly without any adverse complication during treatment. The reason why the study showed a significant reduction may be because the most suitable strain was selected. *L. crispatus* is readily isolated from the vagina of healthy women [12,13] and is nearly universal in its ability to produce hydrogen peroxide and to bind to vaginal epithelial cells. In the present study, the hydrogen peroxide production of three *L. crispatus* strains was measured, resulting in the selection of strain GAI 98332 because it produced the highest level of hydrogen peroxide.

Although antimicrobial agents are quite effective at providing a clinical cure for UTI, they do not change a patient's susceptibility to recurrences. The use of antimicrobial agents results in yeast vaginitis as a result of the disruption of the normal levels of intestinal and vaginal flora and the development of antimicrobial resistance. In fact, resistance rates to trimethoprim-sulfamethoxazole in the USA and Canada and to fluoroquinolones in Spain among *E. coli* isolates are both 18% [14–16]. Current societal trends in healthcare show a definite movement toward the use of natural remedies and away from chemotherapeutic regimens [2]. Therefore, a new strategy for the prophylaxis of recurrent UTI in women should be developed. In such cases, it has been noted that probiotics, which are defined as living microorganisms that, when administered in adequate amounts, confer a health benefit to the host [3], can be used as an alternative preventative approach.

It is believed that the pathogens of UTI ascend from the rectum to the vagina and then to the urinary bladder. This process is mediated by bacterial adherence and is not altered

by antibiotic use [17]. This leads to the investigation that the colonization of pathogens in the vagina is important for recurrent cystitis to occur. In fact, the flora of the urogenital tract is abnormal in patients with recurrent UTI compared with those of healthy women [4–6]. The use of probiotics to restore the normal vaginal flora and provide a competitive bacterial barrier is becoming increasingly acceptable.

Lactobacilli are Gram-positive rods; primarily facultative or strict anaerobes that generally have a fastidious growth requirement. They prefer an acidic environment and help create one by producing lactic and other acids. Generally, lactobacilli have not been associated with disease, and for more than 100 years have been regarded as non-pathogenic members of the intestinal and urogenital floras [18].

The role of lactobacilli in the maintenance of vaginal health was first recognized by Doederlein in the late 18th century [19]. Thus, the concept of providing exogenous *Lactobacillus* for maintaining optimal vaginal microflora is more than a century old. Vaginal lactobacilli protect the female urogenital tract from pathogen colonization and, therefore, can contribute to the prevention of genitourinary tract infection. Many studies have been published describing the relationships between bacterial vaginosis and lactobacilli [20–22]. Studies utilizing *Lactobacillus*-containing products such as yoghurt have been largely unsuccessful in demonstrating successful colonization with the exogenous strain and influencing clinical outcomes related to bacterial vaginosis [23]. However, it has been reported that *Lactobacillus* vaginal capsules are effective in treating bacterial vaginosis and in re-establishing a *Lactobacillus*-predominant flora [24]. In addition, there are some publications on the preventative effect of *Lactobacillus* vaginal suppositories on recurrent UTI. Reid et al. have demonstrated that *Lactobacillus* vaginal suppositories are safe and may be effective in reducing the recurrence of UTI [10]. Conversely, Baerheim et al. have demonstrated that uncertainty exists as to whether the vaginal application of lactobacilli reduces the infection rate in cystitis-prone women [11]. Since the effectiveness of *Lactobacillus* vaginal suppositories on recurrent UTI is still inconclusive, a prospective clinical study to confirm the safety and effectiveness of *Lactobacillus* vaginal suppositories against recurrent UTI was performed.

The concept of probiotics is simple in some ways, but the critical issues are the selection of the optimal strains, verification of the necessity for, and mechanisms of, certain factors in the interference with pathogens, and clinical proof that there is merit to the theory [5]. In general terms, protective roles of vaginal lactobacilli have been identified for lactobacilli to be effective probiotic organisms. The features of lactobacilli are: (i) the ability to produce antimicrobial compounds such as lactic acid, bacteriocins, hydrogen peroxide, etc. [25–28]; (ii) the competitive exclusion of genitourinary pathogens [29,30]; (iii) the ability to produce a biosurfactant that inhibits the adhesion of uropathogens to surfaces [27,31]; and (iv) the non-specific augmentation of the innate immune system [32]. Of these features, the present study focused on hydrogen peroxide production. Lactobacilli and the hydrogen peroxide that they produce are increasingly recognized as essential components of a healthy microflora environment. Hydrogen peroxide is toxic to many microorganisms at concentrations that are typical in the vaginal fluid, and thus provides an intrinsic protective mechanism in the vagina [33,34]. In addition, recent studies have established that *L. crispatus* and *L. jensenii* are two of the most readily isolated microorganisms from vaginal flora [12,13].

In conclusion, the data of the present study suggest that the administration of vaginal suppositories containing *L. crispatus* GAI 98332 may be a safe and promising strategy for the prevention of recurrent UTI. It is recommended that future studies focus on determining the optimal dosage, duration and mode of lactobacilli delivery for establishing vaginal and/or periurethral colonization. Furthermore, it is proposed that prospective randomized studies be performed.

Acknowledgments

We thank Ritsuko Mitsuhata, Kensuke Shimizu and Kazunori Matsuda for their technical assistance. This work was supported by a Grant-in-Aid for Exploratory Research (2001–2003, No. 13877267) from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

References

[1] Reid G, Bruce AW, Cook RL, Llano M. Effect on urogenital flora of antibiotic therapy for urinary tract infection. Scand J Infect Dis 1990;22:43–7.
[2] Reid G. Potential preventive strategies and therapies in urinary tract infection. World J Urol 1999;17:359–63.
[3] Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria, a joint FAO/WHO expert consultation. October 1–4 2001; Cordoba, Argentina. http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf.
[4] Stamey TA. The role of introital enterobacteria in recurrent urinary infections. J Urol 1973;109:467–72.
[5] Schaeffer AJ, Stamey TA. Studies of introital colonization in women with recurrent urinary infections. IX. The role of antimicrobial therapy. J Urol 1977;118:221–4.
[6] Seddon JM, Bruce AW, Chadwick P, Carter D. Introital bacterial flora—effect of increased frequency of micturition. Br J Urol 1976;48:221–8.
[7] Reid G, Chan RC, Bruce AW, Costerton JW. Prevention of urinary tract infection in rats with an indigenous Lactobacillus casei strain. Infect Immun 1985;49:320–4.
[8] Reid G, Bruce AW, Fraser N, Heinemann C, Owen J, Henning B. Oral probiotics can resolve urogenital infections. FEMS Immunol Med Microbiol 2001;30:49–52.
[9] Reid G. Probiotic agents to protect the urogenital tract against infection. Am J Clin Nutr 2001;73(Suppl. 2):S437–43.
[10] Reid G, Bruce AW, Taylor M. Influence of three-day antimicrobial therapy and *lactobacillus* vaginal suppositories on recurrence of urinary tract infections. Clin Ther 1992;14:11–6.

[11] Baerheim A, Larsen E, Digranes A. Vaginal application of lactobacilli in the prophylaxis of recurrent lower urinary tract infection in women. Scand J Prim Health Care 1994;12:239–43.
[12] Antonio MA, Hawes SE, Hillier SL. The identification of vaginal *Lactobacillus* species and the demographic and microbiologic characteristics of women colonized by these species. J Infect Dis 1999;180:1950–6.
[13] Bonadio M, Meini M, Spitaleri P, Gigli C. Current microbiological and clinical aspects of urinary tract infections. Eur Urol 2001;40:439–44.
[14] Reid G, Seidenfeld A. Drug resistance amongst uropathogens isolated from women in a suburban population: laboratory findings over 7 years. Can J Urol 1997;4:432–7.
[15] Gupta K, Scholes D, Stamm WE. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. J Am Med Assoc 1999;281:726–38.
[16] Oteo J, Campos J, Baquero F. Spanish members of the European Antimicrobial Resistance Surveillance System. Antibiotic resistance in 1962 invasive isolates of *Escherichia coli* in 27 Spanish hospitals participating in the European Antimicrobial Resistance Surveillance System. J Antimicrob Chemother 2002;50:945–52.
[17] Reid G, Bruce AW, Beheshti M. Effect of antibiotic treatment on receptivity of uroepithelial cells to uropathogens. Can J Microbiol 1988;34:327–31.
[18] Bibel DJ. Elie Metchnikoffs bacillus of long life. Am Soc Microbiol News 1988;54:661–5.
[19] Butler BC, Beakley JW. Bacterial flora in vaginitis: a study before and after treatment with pure cultures of Doederlein bacillus. Am J Obstet Gynecol 1960;79:432–40.
[20] Reid G, Beuerman D, Heinemann C, Bruce AW. Probiotic *Lactobacillus* dose required to restore and maintain a normal vaginal flora. FEMS Immunol Med Microbiol 2001;32:37–41.
[21] Reid G, Burton J. Use of *Lactobacillus* to prevent infection by pathogenic bacteria. Microbes Infect 2002;4:319–24.
[22] Reid G, Howard J, Gan BS. Can bacterial interference prevent infection? Trends Microbiol 2001;9:424–8.
[23] Colodner R, Edelstein H, Chazan B, Raz R. Vaginal colonization by orally administered *Lactobacillus rhamnosus* GG. Isr Med Assoc J 2003;5:767–9.
[24] Reid G, Charbonneau D, Erb J, et al. Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. FEMS Immunol Med Microbiol 2003;35:131–4.
[25] Hillier SL, Krohn MA, Klebanoff SJ, Eschenbach DA. The relationship of hydrogen peroxide-producing lactobacilli to bacterial vaginosis and genital microflora in pregnant women. Obstet Gynecol 1992;79:369–73.
[26] McGroarty JA, Reid G. Detection of a *Lactobacillus* substance that inhibits *Escherichia coli*. Can J Microbiol 1988;34:974–8.
[27] Verlaeds MM, van der Mei HC, Reid G, Busscher HJ. Inhibition of initial adhesion of uropathogenic *Enterococcus faecalis* by biosurfactants from *Lactobacillus* isolates. Appl Environ Microbiol 1996;62:1958–63.
[28] Reid G, McGroarty JA, Angotti R, Cook RL. *Lactobacillus* inhibitor production against *Escherichia* coli and coaggregation ability with uropathogens. Can J Microbiol 1988;34:344–51.
[29] Reid G, Cook RL, Bruce AW. Examination of strains of lactobacilli for properties that may influence bacterial interference in the urinary tract. J Urol 1987;138:330–5.
[30] Hawthorn LA, Reid G. Exclusion of uropathogen adhesion to polymer surfaces by *Lactobacillus acidophilus*. J Biomed Mater Res 1990;24:39–46.
[31] Velraeds MM, van de Belt-Gritter B, van de Mei HC, Reid G, Busscher HJ. Interference in initial adhesion of uropathogenic bacteria and yeasts to silicone rubber by a *Lactobacillus acidophilus* biosurfactant. J Med Microbiol 1998;47:1081–5.
[32] Reid G. The scientific basis for probiotic strains of *Lactobacillus*. Appl Environ Microbiol 1999;65:3763–6.
[33] Eschenbach DA, Davick PR, Williams BL, et al. Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women with bacterial vaginosis. J Clin Microbiol 1989;27:251–6.
[34] Beigi RH, Wiesenfeld HC, Hillier SL, Straw T, Krohn MA. Factors associated with absence of H_2O_2-producing *Lactobacillus* among women with bacterial vaginosis. J Infect Dis 2005;191:924–9.