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Preventive Effects of *Lactobacillus* Mixture on Experimental *E. coli* Urinary Tract Infection in Infant Rats

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Purpose: Urinary tract infection (UTI) is an ascending infection of fecal uropathogens, urogenital lactobacilli are suggested to play a role in the prevention of UTI. This study was to investigate whether *lactobacillus* mixture (LM) could prevent the experimental infantile UTI. **Materials and Methods:** The LM were composed of three *lactobacillus* strains (L. gasseri, L. rhamnosus, and L. reuteri). Mother rats were grouped as *lactobacillus* (LB) group I (LB I, n=22), II (LB II, n=24) and control (n=20). LB I and LB II were fed with LM (1 mL/day) and control with phosphate-buffered saline (PBS) from late pregnancy through lactation. All newborn rats were breast-fed and their urine and stool were collected at the end of the 3rd week to compare *lactobacillus* colony. Then, infant rats from LB II were treated with intravesical instillation of LM. Infant rats from LB I and control were instilled with PBS. Twenty-four hours later, experimental UTI was introduced by intravesical instillation of standard *E. coli* strain. After 72 hours later, the infant rats were sacrificed for histologic examination. **Results:** Lactobacilli colonies in urine and stool were not statistically different among the three groups. The incidence of pyelonephritis in the LB II was 16.7% (4/24), LB I 72.7% (16.22) and control 75.0% (15/20) (p=0.015). The incidence of cystitis was not significantly different among the three groups. **Conclusion:** The intravesically instilled LM significantly prevented experimental pyelonephritis in infant rats, however, LM administered orally to the pregnant and lactating mother rats did not.

Key Words: Lactobacillus, intravesical instillation, cystitis, pyelonephritis

INTRODUCTION

Urinary tract infection (UTI) is the most common bacterial infection in infants and has been documented as an ascending infection of the patient's own fecal uropathogens, evidenced by genomic profiling study.[1] Therefore, the role of *lactobacillus*, the most dominant urogenital microflora, has been a focus in preventing UTI.[2,3]

Intraurethrally instilled *lactobacillus* strains were proven to prevent experimental UTI in adult animal models,[4-6] and clinical application of *lactobacillus* suppositories showed beneficial effects in reducing the recurrence rate of UTI in adult women.[7-9] However, the preventive effect of oral *lactobacillus* probiotics against

UTI has not sufficiently been investigated in adults.[10,11]

In early infancy when the UTI incidence is extremely high, postnatal development of genitourinary *lactobacilli* is considered very important in preventing UTI. It was confirmed that some maternal *lactobacilli* are transferred to their infants during birth and through breast milk.[12-14] Probiotic *lactobacilli*, administered to lactating mother rats, were also proven to be transferred to their infant guts[15,16] and then to genitourinary tract.[17] However, a question of whether this mother to infant transmission prevents infantile UTI has not been clarified.

Therefore, we evaluated whether *lactobacilli* orally administered to pregnant and lactating mother rats could prevent experimental UTI of their infant rats, and compared the effects with those of intravesically instilled *lactobacilli* to infant rats.

MATERIALS AND METHODS

Materials

Lactobacillus mixture

For this study, we selected three *lactobacillus* strains (*L. gasseri*, *L. rhamnosus*, *L. reuteri*), which were isolated from the healthy infant feces and mixed. For optimal dosage, the *lactobacillus* mixture (LM) was incubated in the DeMan-Rogosa-Sharpe (MRS) agar medium to make six different dosages [10^6-10^{11} colony forming unit (CFU)/mL], which were tested for the antimicrobial activities against standard *E. coli* strain (ATCC No. 25922, Seattle, WA, USA). We selected the dosage 10^{10} CFU/mL, whose antimicrobial activity was maximal (Fig. 1).

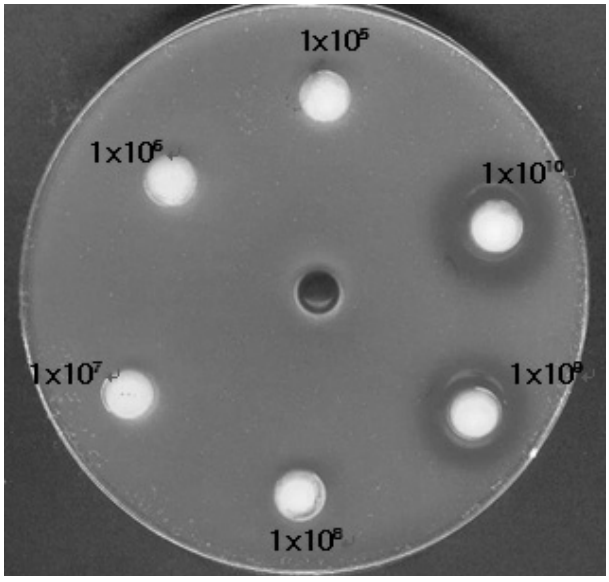

Fig. 1. Inhibitory zone (diameter, mm) at different dosage of lactobacilllus mixture against standard *E. coli* (ATCC 25922). The optimal dosage (CFU/mL) for maximal inhibition was dertermined as 1×10^{10} CFU/mL.

The experimental infant rats

Forty-eight breast-fed Sprague Dawley infant rats (Seoul, Korea), whose mother rats were fed the LM (1 mL/day) via gavage tube from late pregnancy through lactation, were evenly allocated to the *lactobacillus* (LB) I (n=24) or II group (n=24). The control group included 20 infant rats, whose mother rats were given phosphate-buffer solution (PBS, 1 mL). All infant rats were breast-fed for 3 weeks, when the same LM (1 mL) was intravesically instilled to infant rats of the LB II group and PBS to those of the LB I and the control group. The study protocol was approved by the ethical committee of the hospital.

Urine and stool culture for lactobacillus

At age 3 weeks of infant rats, urines and stools were collected. Fecal specimens were placed to a 50 mL test tube together with sterile saline and shaken for 1 minute. For *lactobacillus* culture, urines and diluted fecal supernatants (200 μL) were inoculated into *lactobacillus*-selective MRS agar (Oxoid, Basingstroke, UK) and incubated anaerobically at 37°C for 48 hours. *Lactobacillus* was confirmed by Gram-positive white, smooth bacillus, and colonies (CFU/mL) were counted.

Intravesical instillation of the lactobacillus mixture

After completion of stool and urine collection, the same LM (1 mL), given to the mother rats, was instilled into the bladder of infant rats of the LB II group using a 16-gauge silicone catheter after ketamine anesthesia. PBS (1 mL) was instilled into LB I and the control group.

Induction of experimental E. coli UTI

Twenty-four hours after intravesical instillation of the LM or PBS, 1 mL of standard *E. coli* strain (10^7 CFU/mL) was instilled into the bladder of infant rats to induce experimental *E. coli* UTI through a 16-gauge silicone catheter after ketamine anesthesia.

Histopathological examination

Seventy-two hours after intravesical instillation of *E. coli*, the infant rats were sacrificed for histopathologic examination. Both kidneys and bladder were extracted and fixed for

24 hours in 10% buffered formalin solution. The kidney was embedded in paraffin, sectioned 3 μm in thickness using a rotatory microtome, and Hematoxylin-Eosin staining and Masson-Trichrome staining were performed. According to the histopathological changes, cystitis and pyelonephritis were diagnosed and the incidence was compared. For semiquantative evaluation, the severity of inflammation in cystitis and pyelonephritis was scored. The cystitis score ranged from 0 to 3 (0 point without inflammatory cells, 1 point with a few inflammatory cells in the submucosa, 2 points with more than 5 focal inflammatory cells in the submucosa, and 3 points with diffuse inflammatory cells in the submucosa). The pyelonephritis score ranged from 0 to 4 (0 point without inflammation, 1 point with occasional inflammation in the pelvic mucosa, 2 points with continuous inflammation along the pelvic mucosa, 3 points with focal inflammation from the pelvic mucosa to the renal medulla, and 4 points with diffuse inflammation to the renal medulla).

Statistical methods

For statistical analysis, SPSS version 11.5 (SPSS Inc., Chicago, IL, USA) was used. ANOVA test was performed for comparison of the number of *lactobacillus* or *E. coli*, histopathological findings, the incidence and the score of cystitis and pyelonephritis between LB I, II and control groups. For the comparison of the number of *lactobacillus* and *E. coli* CFU/mL after experimental *E. coli* UTI, Wilcoxon signed rank test was used. p value less than 0.05 was determined to be statistically significant.

RESULTS

Lactobacillus colonization status in stools and urines of infant rats

The numbers of *lactobacillus* CFUs in the stools of infant rats were $3.5\times10^{8}\pm6.6\times10^{7}$ CFU/mL in the LB I group, $4.1\times10^{8}\pm2.5\times10^{8}$ CFU/mL in the LB II group, and $1.7\times10^{7}\pm7.3\times10^{6}$ CFU/mL in the control group ($p>0.05$). The numbers of *lactobacillus* CFUs in the urines of infant rats were 2930±314.4 CFU/mL in the LB I group, 5892±370.5 CFU/mL in the LB II group, and 1658±361.1 CFU/mL in the control group ($p>0.05$) (Fig. 2).

The incidence of experimental UTI

The incidence of cystitis was 95.5% (21/22) in the LB I group, 87.5% (21/24) in the LB II group, and 100% (20/20) in the control group ($p>0.05$). The incidence of pyelone-

Table 1. Incidence of Experimental *E. coli* Urinary Tract Infection in Infant Rats

UTI	*Lactobacillus* I (n=22)	*Lactobacillus* II (n=24)	Control (n=20)
Cystitis	21 (95.5%)	21 (87.5%)	20 (100%)
Pyelonephritis	16 (72.7%)	4 (16.7%)*	15 (75.0%)
Total	21 (95.5%)	21 (87.5%)	20 (100%)

UTI, urinary tract infection.
*p=0.015 vs. control.

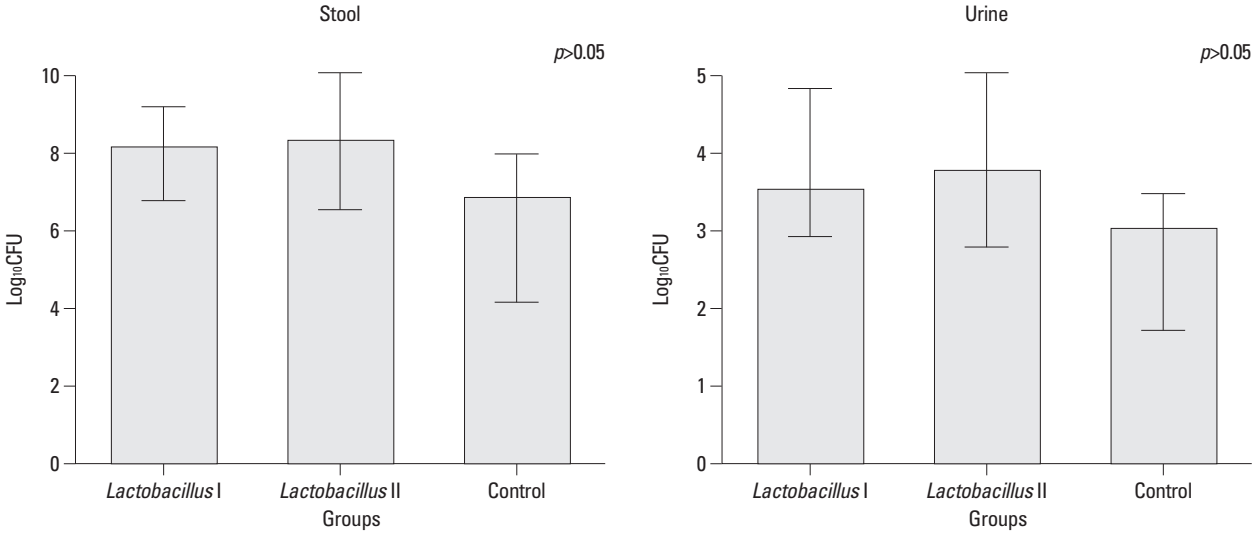

Fig. 2. Stool and urine *lactobacillus* colonization in *lactobacillus* I, II and control group.

phritis was 16.7% (4/24) in the LB II group, which was significantly lower than 75.0% (15/20) of the control group (p=0.015) (Table 1).

The semiquantitative inflammatory score of cystitis and pyelonephritis

The cystitis score of the LB II group (1.25±0.98) was lower than those of the LB I group (1.5±0.78) and control group (1.8±1.06) (p>0.05) (Fig. 3). The pyelonephritis score of the LB II group (0.33±0.68) was significantly lower than those of the LB I group (1.31±1.21) and control group (1.95±1.72) (p=0.006) (Fig. 3).

DISCUSSION

In preventing infantile UTI, postnatal development of genitourinary *lactobacilli* is considered very important. Indeed, *lactobacilli* in the maternal vagina are the first source of *lactobacilli* of newborn infants. While passing through the birth canal, maternal vaginal *lactobacilli* are transferred to the sterile neonate's gut for the first time.[12] *Lactobacilli* in breast milk are the second important source of infant's gut *lactobacilli*.[13] Approximately 6 days after birth, the number of CFU/mL of *lactobacillus* in the feces of breast-fed infants was 1000 times more than that of enterobacteriae, but 10 times less in the feces of bottle-fed infants.[14] Probiotic *lactobacilli*, supplied during pregnant and lactating period, were proved to colonize the infant's gut.[15,16] These vertically transmitted *lactobacilli* are transferred from gut to genitourinary tract.[17]

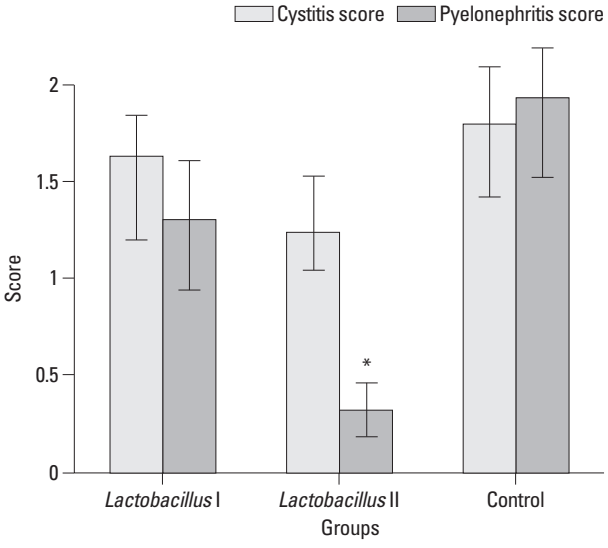

Fig. 3. Cystitis and pyelonephritis score in experimental *E. coli* urinary tract infection (cystitis score p>0.05, pyelonephritis score *p=0.006 vs. control).

Many earlier studies demonstrated that the number of urogenital *lactobacilli* is significantly decreased in infants with UTI[18] as well as in woman with urethritis and recurrent UTI.[7-9,19-22] Antimicrobial activities of *lactobacillus* strains against uropathogens have been studied in many *in vitro* tests.[23-26] *Lactobacillus* strains imped the adherence of uropathogens by secreting biosurfactants,[23] compete with uropathogens in the binding site on vaginal epithelial cells,[24] and inhibit the growth of uropathogens by secreting hydrogen peroxide, lactate, bacteriocin, and other antimicrobial molecules.[25] They also enhance the local immunity of the intestinal mucosa, and improve the innate immunity and cell-mediated immunity by activating monocytes.[26]

The preventive effects of intravesically instilled *lactobacilli* against UTI have been proven in adult animal models with different strains and different dosages.[4-6] When *L. casei* GR1 (5×10^9 CFU/mL), isolated from healthy adult women, was intravesically instilled to adult rats and was then swabbed twice weekly for 21 days onto the introitus before challenge with an uropathogen suspension (*E. coli*, *K. pneumoniae*, *P. aerusinosa*), experimental UTI was prevented in 84% of the animals.[4] *L. casei* shirota strain (1×10^8 CFU/day), when administered intraurethrally to a mouse 24 hours prior to the induction of experimental *E. coli*, dramatically inhibited *E. coli* growth and inflammatory responses in the urinary tract.[5] Furthermore, intraurethral instillation of the indigenous *L. murinus* strain (1×10^8 CFU/mL) also significantly prevented *Proteus mirabilis* ascending UTI in a mouse model.[6]

In the present study, intravesically instilled *lactobacilli* to infant rats showed the significant preventive effect against experimental pyelonephritis, whereas orally administered *lactobacilli* to pregnant and lactating mother rats did not prevent the infection of their infant rats. This might be due to insufficient increase of *lactobacillus* colonization in their stools and urines. Further studies are necessary to find ideal *lactobacillus* strains and an optimal oral dosage that are compatible with preventive effect of intravesically instilled *lactobacillus*.

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