Preventive Effects of \textit{Lactobacillus} Mixture on Experimental \textit{E. coli} Urinary Tract Infection in Infant Rats

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\textbf{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 \textit{lactobacillus} mixture (LM) could prevent the experimental infantile UTI. \textbf{Materials and Methods:} The LM were composed of three \textit{lactobacillus} strains (\textit{L. gasseri}, \textit{L. rhamnosus}, and \textit{L. reuteri}). Mother rats were grouped as \textit{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 \textit{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 \textit{E. coli} strain. After 72 hours later, the infant rats were sacrificed for histologic examination. \textbf{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 (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 \textit{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 \textit{E. coli} strain. After 72 hours later, the infant rats were sacrificed for histologic examination. \textbf{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. \textbf{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.

\textbf{Key Words:} Lactobacillus, intravesical instillation, cystitis, pyelonephritis

\section*{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.\textsuperscript{1} Therefore, the role of \textit{lactobacillus}, the most dominant urogenital microflora, has been a focus in preventing UTI.\textsuperscript{2-3}

Intraurethrally instilled \textit{lactobacillus} strains were proven to prevent experimental UTI in adult animal models,\textsuperscript{4,5} and clinical application of \textit{lactobacillus} suppositories showed beneficial effects in reducing the recurrence rate of UTI in adult women.\textsuperscript{6-9} However, the preventive effect of oral \textit{lactobacillus} probiotics against
UTI has not sufficiently been investigated in adults. 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. Probiotic lactobacilli, administered to lactating mother rats, were also proven to be transferred to their infant guts and then to genitourinary tract. 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 10^9 CFU/mL, whose antimicrobial activity was maximal (Fig. 1).

**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

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Fig. 1. Inhibitory zone (diameter, mm) at different dosage of lactobacillus mixture against standard E. coli (ATCC 25922). The optimal dosage (CFU/mL) for maximal inhibition was determined as 1×10^9 CFU/mL.
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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 semiquantitative 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).

**RESULTS**

*Lactobacillus* colonization status in stools and urines of infant rats

The numbers of *lactobacillus* CFUs in the stools of infant rats were $3.5 \times 10^8 \pm 6.6 \times 10^7$ CFU/mL in the LB I group, $4.1 \times 10^8 \pm 2.5 \times 10^8$ CFU/mL in the LB II group, and $1.7 \times 10^7 \pm 7.3 \times 10^6$ CFU/mL in the control group ($p>0.05$). The numbers of *lactobacillus* CFUs in the urines of infant rats were $2930 \pm 314.4$ CFU/mL in the LB I group, $5892 \pm 370.5$ CFU/mL in the LB II group, and $1658 \pm 361.1$ 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 pyelonephritis was 72.7% (16/22) in the LB I group, 16.7% (4/24) *p=0.015* vs. control in the LB II group, and 75.0% (15/20) in the control group ($p>0.05$). The incidence of 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.

### 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.
Lactobacillus Control

infection (cystitis score 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).

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 genital urinary 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. Lactobacilli in breast milk are the second important source of infant’s gut lactobacilli. 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. Probiotic lactobacilli, supplied during pregnant and lactating period, were proved to colonize the infant’s gut. These vertically transmitted lactobacilli are transferred from gut to genital urinary tract.

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

The preventive effects of intravesically instilled lactobacilli against UTI have been proven in adult animal models with different strains and different dosages. When L. casei GR1 (5×10⁶ 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. aeruginosa), experimental UTI was prevented in 84% of the animals. L. casei shirotia strain (1×10⁶ CFU/day), when administered intrareurally 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. Furthermore, intraurethral instillation of the indigenous L. murinus strain (1×10⁶ CFU/mL) also significantly prevented Proteus mirabilis ascending UTI in a mouse model.

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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