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Complete List of Authors:	Otsuki, Katsufumi; Showa University Koto Toyosu Hospital, Obstetrics and Gynecology Imai, Noriaki; Hachinohe City Hospital, Obstetrics and Gynecology
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Effects of lactoferrin in six patients with refractory bacterial vaginosis

Katsufumi Otsuki¹⁾, Noriaki Imai²⁾

¹⁾ Department of Obstetrics and Gynecology, Showa University Koto Toyosu Hospital

²⁾ Department of Obstetrics and Gynecology, Hachinohe City Hospital

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Corresponding Author:

Katsufumi Otsuki

Department of Obstetrics and Gynecology, Showa University Koto Toyosu Hospital

5-1-38 Toyosu, Koto-ku

Tokyo 135-8577

Tel: 03-6204-6000

Fax: 03-6204-6988

E-mail: otsuki@med.showa-u.ac.jp

Abstract

We previously reported that Lactoferrin (LF) could most likely be effective for preventing preterm delivery and intrauterine infections based on data derived from mice and rabbits. Here we describe six women with a history of multiple pregnancy losses or preterm delivery and refractory bacterial vaginosis, who received prebiotic LF therapy and delivered an infant normally. Five of the women were pregnant and one was not at the time of this study. The Ethics Committee at Showa University Hospital and Showa University Koto Toyosu Hospital approved the therapeutic protocol.

Vaginal suppositories and oral prebiotic LF were administered to patients who were refractory to conventional treatment for vaginosis and had a history of late miscarriages and very early preterm delivery due to refractory vaginitis and chorioamnionitis. Lactoferrin significantly improved the vaginal bacterial flora. *Lactobacillus* that was detectable in the vaginas of all patients after one month of LF therapy gradually became dominant.

The findings from these six patients suggest that administering LF to humans could help to prevent refractory vaginitis, cervical inflammation and preterm delivery.

Introduction

Human milk and neutrophils contain large amounts of the glycoprotein LF which is a prebiotic in humans. LF also has been reported to be a biologic therapeutic protein for the treatment of diarrhea, inflammatory bowel diseases, anemia, wound healing, sepsis and cancer. We previously reported a high likelihood that LF could help to prevent preterm delivery and intrauterine infections in humans based on evidence derived from studies of mice and rabbits.¹⁻⁴

Here, we describe six patients with a history of pregnancy losses or refractory bacterial vaginosis among whom five who were treated with LF throughout pregnancy proceeded to full term and delivered healthy infants normally.

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Case reports

Five of six women (age, 30 – 39 years) with a history of several pregnancy losses or preterm delivery and refractory bacterial vaginosis, who received LF therapy delivered normally and one was not pregnant. Two of the women started LF therapy before becoming pregnant and the others started therapy from 11 to 21 weeks of gestation when refractory bacterial vaginosis was diagnosed. All patients had high-risk factors for preterm delivery including a history of delivery before 30 weeks of gestation. They also had abnormal bacterial flora and bacterial vaginosis. Vaginal *Lactobacillus* was either absent or very scarce before LF administration. Therefore, we obtained ethical approval from our Institutional Review Board (IRB) and written informed consent from each patient to administer vaginal suppositories (150 mg/day) in the evening after shower or bath and oral tablets (700 mg/day) after breakfast of prebiotic bovine LF (Shimizu 2004) (Ono et al.) (NRL Pharma, Kawasaki, Japan). The degree of LF purity was 93.6%, LPS contamination was less than 10 EU/ml, and iron saturation was 11% of the oral LF and the LF used in the suppository. Bacterial flora and culture of vagina was done every two weeks after LF administration. *Lactobacillus* that appeared after one month of LF

gradually became dominant and the patients became pregnant around three months later. The patients continued with the oral and vaginal administration of LF until delivery. Cervical maturation related to preterm delivery did not arise and the bacterial flora of the vagina remained normal. *Lactobacillus* remained dominant throughout pregnancy and the course was uneventful with no adverse events affecting either the women or their infants.

Discussion

The rate of preterm delivery is 5.7% in Japan, which is one of the lowest among advanced countries and half that in the USA. Although the frequency of preterm labor is low in Japan, further efforts are underway to further reduce it. Rates of perinatal and early neonatal mortality continue to decrease in Japan, whereas those of preterm births are gradually increasing for reasons that remain unknown, although an increased frequency of maternal complications associated with having children later in life, myoma uteri, pregnancy-induced hypertension, other complications associated with aging and an increased need for in vitro fertilization and other reproductive techniques might be involved. Vaginal lavage has been linked to an increasing risk of PID.

However, most of these reports describe vaginal lavage implemented by patients at home (Cottrell), instead of in hospital, where lavage is usually administered by medical staff in Japan. One report (Luong et al.) and our unpublished data have linked vaginal lavage to the extension of pregnancy among patients in preterm labor at MFICUs in Japan.

Many maternal or fetal genetic, endocrine and immune factors, stress and nutritional status are involved in preterm labor. Such factors can work alone or together to disrupt the mechanisms that maintain pregnancy and result in preterm delivery. Therefore, women at risk for preterm labor and birth should be identified during the first trimester.

Intrauterine infections might trigger preterm delivery in humans via locally produced cytokines such as interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF)- α , which induce the production of cyclooxygenase (cox)-2, that in turn accelerates the production of prostaglandin (PG) E₂ and F₂ α . Such cascades ultimately cause ripening of the uterine cervical canal, the generation of uterine contractions and ultimately, preterm delivery (Romero et al. 1993). We previously infused *E. coli* directly into the uterine cervical canals of rabbits to prepare models with a very similar etiology to that of

preterm delivery in humans, and studied the ability of LF to prevent preterm delivery. Lactoferrin extended the gestational period and decreased fetal mortality rate in rabbit models, and inhibited the production of inflammatory TNF- α in maternal serum and amniotic fluid (Mitsuhashi et al. 2000) (Hasegawa et al. 2005). Lactoferrin also inhibited cervical maturation in experimental animal models of preterm delivery (Yakuwa et al. 2007) (Nakayama et al. 2008). These results suggested that LF could prevent and treat preterm delivery and improve the prognosis of newborns¹⁻⁴. Machnicki *et al.* (Machnicki et al. 1993) reported that the intravenous LF decreased serum concentrations of IL-6 and TNF- α in mice. Others have also suggested that LF can prevent iron-deficiency anemia and associated preterm delivery in humans (Giunta et al. 2012; Paesano et al. 2012). These findings indicate that supplementary LF, which inhibits inflammatory cytokines, could help to prevent preterm delivery induced by infection. Furthermore, LF neutralizes mycotoxins and reduces TNF- α concentrations in patients with sepsis, and thus might have potential to act as a therapeutic agent against sepsis (Zhang et al. 1999). In the near future, randomized clinical trials with LF suppositories and oral LF (as was used in the present study) vs LF suppositories alone

should be conducted to determine whether ingestion of LF in addition to a LF vaginal suppository is required to obtain a beneficial effect on preterm delivery.

Conclusions

We administered LF to six patients with refractory bacterial vaginosis and recurrent preterm delivery and all of five who were pregnant achieved the desired outcome of a normal delivery without complications. The mechanisms of LF relative to inflammation and infection are varied, and a description is currently difficult to integrate even with reference to reports by various authors. Despite the limited number of patients in the present study, the findings suggest that LF can help to prevent refractory vaginitis, cervical inflammation and preterm delivery in humans. The oral and cervical administration of recombinant human lactoferrin extended the duration of gestation and increased the survival rate of fetuses after inoculation with bacteria. This might be due to both the anti-inflammatory and anti-bacterial effects of LF. Recombinant human lactoferrin offers promise as an agent to prevent preterm delivery triggered by cervical infection in humans.

More data from larger studies are needed to support this notion. We are

planning future studies that incorporate serial anti-inflammatory marker measurements and a molecular microbiome evaluation. An important ethical issue that can arise when conducting a randomized clinical trial of critical medications is the effect of placebo administration on the patient. In the case of a trial testing the effect of LF on preterm delivery, being administered a placebo may result in preterm delivery and enormous harm to the fetus. Such effects must be carefully considered, with the wellbeing of the patient being placed ahead of the statistical power of the trial, and if necessary, the placebo arm of a trial may need to be omitted.

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Table 1 Effects of oral and vaginal lactoferrin in two patients

Age (y)	Medical history	Shirodkar cervical cerclage	Time of LF start	Route of LF administration*	Cultured vaginal discharge		Gestational week of delivery	Birth weight (g)	Neonatal gender
					Before LF	After 3 m of LF			
39	Preterm delivery at 19, 13 and 25 weeks gestation	Yes	Before pregnancy	O and V	<i>GBS</i> 3+ <i>G vaginalis</i> 1+ <i>CNS</i> 1+	Lact 3+	38 Cesarean section	2879	Male
30	Two cervical conizations	Yes	21gw	O and V	Lact 1+ <i>E. coli</i> 3+ <i>α Hemo Strept</i> 2+	Lact 2+ <i>E. coli</i> 1+	35 Cesarean section	2391	Female

*Oral LF, 700 mg/day; vaginal LF suppositories, 90mg/day

O, orally; V, vaginally

gw, gestational weeks

m, months

α Hemo Strept, alpha hemolytic Streptococcus; *CNS*, coagulase negative Staphylococcus; *E. coli*, Escherichia coli; *GBS*, Group B Streptococcus; *G vaginalis*, Gardnerella vaginalis;

Lact, Lactovacillus,

Table 2 Oral lactoferrin administrates to four patients

Age (y)	Medical history	Shirodkar cervical cerclage	Time of LF start	Route of LF administration*	Cultured vaginal discharge		Gestational week of delivery	Birth weight (g)	Neonatal gender
					Before LF	After 3 m of LF			
39	Preterm delivery at 28 weeks gestation	No	13	O	<i>G vaginalis</i> 1+ CNS 1+	Lact 1+	38 Cesarean section	2789	female
36	Preterm delivery at 26 weeks gestation	Yes	19	O	Lact 1+ <i>Kl pneumonia</i> 1+ <i>E. coli</i> 2+ <i>C albicans</i> 1+ <i>Enterococcus</i> 1+	Lact 1+ <i>E. coli</i> 1+	38 Cesarean section	3435	female
35	Preterm delivery at 28 weeks gestation	Yes	11	O	<i>G vaginalis</i> 1+	Lact 1+ <i>Ec faecalis</i> 1+ <i>a Hemo Strept</i> 3+	40 Trans-vaginal	3577	female
30	Preterm delivery at 25 weeks	No	Not pregnant	O	<i>E. coli</i> 3+ <i>G vaginalis</i> 1+	Lact 3+ CNS 1+			

gestation	<i>α Hemo Strept 2+</i>
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*Oral LF, 700 mg/day

O, orally

gw, gestational weeks

m, months

α Hemo Strept, alpha hemolytic *Streptococcus*; *C albicans*, *Candida albicans*; CNS, coagulase negative *Staphylococcus*; *E. coli*, *Escherichia coli*; *Ec faecalis*, *Enterococcus faecalis*; GBS, Group B *Streptococcus*; *G vaginalis*, *Gardnerella vaginalis*; *Kl pneumonia*, *Klebsiella pneumoniae*; *Lact*, *Lactovacillus*,

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