POTENTIAL OF LACTOFERRIN IN THE PREVENTION OF PRETERM DELIVERY

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SUMMARY

Objective: Chorioamnionitis (CAM), an ascending infection from maternal bacterial vaginosis or cervicitis, can cause preterm delivery. We hypothesized that lactoferrin (LF), in particular recombinant human LF (rh-LF), may prevent preterm delivery. We conducted three experiments to demonstrate LF’s ability to prevent preterm delivery.

Materials and Methods: First, we correlated human cervical mucus LF concentration and LF and interleukin-6 (IL-6) concentrations in amniotic fluid to gestational weeks, in mothers with and without CAM. Second, using an amnion cell culture system, we studied the preventive effect of rh-LF on inflammatory cytokine production induced by lipopolysaccharide. Using human cervical gland cell lines, we studied the preventive effects of rh-LF on the growth of Escherichia coli. Thirdly, we studied the preventive effect of rh-LF in mouse and rabbit models of preterm delivery. We also examined LF’s preventive effect on the production of inflammatory cytokines in maternal serum and amniotic fluid.

Results: In mothers with CAM, amniotic fluid LF concentrations were elevated and IL-6 production increased as fetal LF levels and inflammation increased. LF administration prevented IL-6 production, demonstrating LF’s anti-cytokine action and control over E. coli. Length of pregnancy was extended and fetal survival rates were higher in mothers who received LF.

Conclusion: It appears that LF is highly beneficial in the prevention of preterm delivery and improving poor fetal prognosis due to elevated inflammatory cytokine levels. Specifically, rh-LF may prevent the production of inflammatory cytokines under E. coli infection or control maternal cytokine production. [Taiwanese J Obstet Gynecol 2005;44(2):123–127]

Key Words: chorioamnionitis, lactoferrin, preterm delivery

Introduction

There is little debate that chorioamnionitis (CAM), which is an ascending infection from bacterial vaginosis and cervicitis, results in preterm delivery [1]. As there are many reports of CAM-induced preterm delivery, it is conceivable that the key to preterm delivery prevention...
The presence of LF modifies the body’s immune function and defends against bacterial invasion. Specifically, LF binds to intracellular membrane proteins and controls bacterial proliferation by binding the available iron needed for bacterial development, thus exerting an effective antibacterial action by destroying bacterial cell membranes. LF also assists the specialization of natural killer cells and lymphocytes, and in phagocytosis by leukocytes and macrophages. LF is discharged from leukocytes to the site of infection, controlling migration and vitalization by adjusting cytokine production. LF controls phagocyte oxygen production and subsequent oxidative injury.

In order to determine LF’s potential in the prevention of preterm delivery, we examined the following: LF’s movement in cervical mucus, to determine its clinical efficacy; the antibacterial and anti-cytokine effects of LF in human cell culture systems; and the preventive effects of recombinant human LH (rh-LF) on preterm delivery (Figure 1).

**LF Production and Secretion in Human Cervical Mucus and Amniotic Fluid**

We examined the presence of LF in cervical mucus and amniotic fluid. To collect the cervical mucus and amniotic fluid, aprotinine, sodium azide (NaN₃), and phosphate-buffered saline (PBS) were added and then anti-human LF IgG polyclonal antibody was used. Changes associated with the specific gestational week were examined. Amniotic fluid samples were also obtained by transabdominal amniocentesis or upon delivery. Twenty-one samples of amniotic fluid (32–39 weeks of gestation) from normal deliveries without amniotic infection were also obtained as controls. Cervical mucus and amniotic fluid samples were centrifuged and the supernatant was stored at −80°C until assay using a sandwich enzyme-linked immunosorbent assay (ELISA). Inter- and intra-assay coefficients of variation were 4.7% and 7.0%, respectively. This study was approved by the ethics committee, and patients gave consent before samples were obtained. Similarly, animal experiments were conducted under the approval of the animal experiment control committee.

LF concentration in cervical mucus from controls was 8.0 ± 11.0 μg/mL, from patients with bacterial vaginosis was 16.3 ± 13.5 μg/mL, and from patients with cervicitis was 15.7 ± 13.4 μg/mL. LF concentration in the bacterial vaginosis group was significantly lower than that in the control group (*p < 0.05*) (Figure 2). The concentrations of LF and IL-6 in amniotic fluid from CAM cases (*n* = 28) were 8.76 ± 0.65 and 6.92 ± 4.88 μg/mL, respectively, and both were significantly higher than in amniotic fluid from those without CAM (*n* = 31; 0.86 ± 0.81 and 0.34 ± 0.25 μg/mL, respectively; *p < 0.01*). There was a significant positive correlation between LF and IL-6 levels in amniotic fluid (*r* = 0.91, *p < 0.01) (Figure 3).

These results show that human cervical cells produce LF and that this production is accelerated during pregnancy. The LF concentration in cervical mucus from patients with bacterial vaginosis or cervicitis was high, meaning that LF production by cervical cells was reduced by inflammation or infection, thus promoting infection and reducing host defenses. This shows that LF has an anti-inflammatory function. On the other hand, LF production in the newborn is increased by inflammation, suggesting that the amniotic LF concentration was high in the CAM group. In addition, a newborn CAM case had a high salivary LF concentration (data not shown). Thus, LF is involved in the anti-inflammatory reaction of the fetus.
Antibacterial and Anti-inflammatory Cytokine Action of LF

The preventive effect of LF on inflammatory cytokine production in amniotic cell culture and bacterial growth in cervical cell lines were examined.

Amniotic cell culture

Confluent primary amniotic cell cultures (7–10 days) were dispersed using 0.5% trypsin, placed in six-well culture dishes in serum-free Dulbecco’s modified Eagle medium (D-MEM)/F12 containing 1% Ab/Am, and the cells were incubated for 24 hours. Lipopolysaccharide (LPS) and/or LF was then added to the medium. After further incubation, the culture medium was collected and the IL-6 concentration was measured using a human IL-6 ELISA kit (Fuji Revio, Tokyo, Japan) [5–7].

IL-6 production was induced by LPS (100 ng/mL) in cultured amnion cells to levels approximately five times that of the control cells. This induced production was significantly inhibited by LF, depending on the dose ($p < 0.05$). Inhibitory levels were well within physiologic LF concentrations (1 $\mu$g/mL). A similar tendency was observed in all other experiments using amniotic membranes (Figure 4).

Cervical cell lines

Two human cervical epithelial cell lines, ME-180 (a non-mucus producing cell, ATCC HTB-33) and HeLa (a mucus-producing cell, ATCC CCL-2) were used. No therapy was used in these studies. The bacterial pathogen, Escherichia coli (ATCC EC-5), was used for inoculation and then isolated from a human infant. Treatment was added at the time of infection. After 2 hours’ incubation, the supernatant was cultured using standard pour-plate techniques. Thereafter, the cells were disrupted and cultured using the same methods. Treatment with rh-LF was used to examine its antibacterial action towards E. coli [8].

It has been suggested that mucus-producing cervical cells play an important role while non-mucus producing cells do not. In the presence of rh-LF, mucus-producing cervical epithelial cells may play an important role in restricting bacterial growth, since rh-LF controlled E. coli development in mucus-producing cells (Figure 5).

Our results demonstrate the anti-cytokine function of LF in human amnion cell culture. The antibacterial function appeared only in mucus-producing cervical cells. It is thought that LF is activated by cytokine and macrophage production, while it is reduced in the presence of E. coli.

Preventive Effect of LF on Preterm Delivery in Animal Models

Mouse model

We conducted this study under the approval of the animal experiment committee of our institution. Female C3H/HeN Crj mice were pair-mated with male Crj: B6D2F1 mice. On day 15 of gestation, as a model of

Figure 3. Lactoferrin (LF) and interleukin-6 (IL-6) concentrations in amniotic fluid from pregnant women with (+; $n = 28$) and without (−; $n = 31$) chorioamnionitis (CAM). Mean ± standard deviation. *$p < 0.01$ versus CAM(−).

Figure 4. Effect of lactoferrin (LF) on lipopolysaccharide (LPS)-induced interleukin-6 (IL-6) production in amniotic cells. Mean ± standard deviation of three culture dishes. *$p < 0.05$ versus control.
preterm delivery, a 50-μg/kg intraperitoneal injection of LPS was administered twice with a 3-hour interval between injections (2:00 and 5:00 pm). One hour prior to each LPS injection (1:00 and 4:00 pm), an intraperitoneal injection of saline with bovine LF (b-LF) or rh-LF (1 mg/body) was administered. In non-LPS-treated controls, an intraperitoneal injection of saline was administered at four times (1:00, 2:00, 4:00, and 5:00 pm). Body weight and delivery time were recorded. To measure plasma levels of IL-6 and tumor necrosis factor-α (TNF-α), other pregnant mice, prepared as above, were sacrificed 6 hours after the second LPS injection. Blood samples were obtained and analyzed as described previously [9–11].

All non-LPS-treated mice had term delivery at 19 ± 0 gestational days. All LPS-treated mice that did not receive LF had preterm delivery at 1.2 ± 0.4 days after the second LPS injection (16.2 ± 0.4 gestational days). Administration of b-LF or rh-LF to LPS-treated mice significantly prolonged gestation to 17.8 ± 0.3 and 18.2 ± 1.3 gestational days, respectively (p < 0.05). All deliveries occurred without maternal death. All preterm deliveries at less than 17 days’ gestation were stillbirths while, in contrast, those at 17 gestational days or later were live births. Plasma IL-6 concentration was significantly higher in LPS-treated mice that did not receive LF (1,628 ± 115 pg/mL) than in non-LPS-treated mice (497 ± 39 pg/mL; p < 0.05). Administration of b-LF or rh-LF to LPS-treated mice significantly suppressed plasma IL-6 levels to 88 ± 6 and 38 ± 30 pg/mL, respectively (p < 0.05) (Figures 6–8).

Rabbit model
We conducted this study under the approval of the animal experiment committee of our institution. Timed pregnant, New Zealand White rabbits (3–4 kg) were anesthetized with an intramuscular injection of ketamine hydrochloride and inoculated with 0.2 mL of E. coli (107 colony forming units, CFU/body) or saline solution using a sterile polyethylene cannula (1.2 mm outer diameter) and an hysteroscope. The rabbits were randomly assigned to one of three groups: A (n = 3) received sterile saline plus sterile saline, B (n = 8) received sterile saline plus E. coli, and group C (n = 7) received rh-LF (5 mg/body) 2 hours before inoculation with E. coli.
Lactoferrin Prevents Preterm Delivery

Figure 8. Effect of recombinant human lactoferrin (rh-LF) on plasma tumor necrosis factor-α (TNF-α) in pregnant mice. Each column represents plasma TNF-α, 6 hours after the second lipopolysaccharide (LPS) treatment: LPS without LF, n = 12; LPS + bovine LF (b-LF), n = 12; LPS + rh-LF, n = 7; non-LPS-treated, n = 12. Values are expressed as pg/mL. *p < 0.05 versus LPS without LF.

We measured TNF-α concentrations in both maternal serum and amniotic fluid.

In the rabbit experiment, fetal survival rates were 95.7% (group A), 0% (group B) and 32.6% (group C). The survival rate in group C was significantly higher than that in group B (p < 0.01). Pregnancy continued for 7.00 ± 0 days from inoculation in group A, 3.25 ± 0.43 days in group B, and 4.85 ± 1.77 days in group C. Pregnancy duration from inoculation in group C was significantly longer than that in group B (p < 0.05). TNF-α concentrations in serum at birth decreased, although group C did not significantly differ in contrast to group B. TNF-α concentrations in amniotic fluid at birth also decreased, although group C did differ in comparison with group B.

These results clearly show that LF prevents preterm delivery in animals. This effect is due to maternal cytokine production in the amnion, placenta, and other tissues. On the other hand, the fetus death rate among rabbits that received LF in the E. coli-induced preterm delivery model was low. This demonstrates that LF can improve the poor prognosis of a fetus subject to inflammatory cytokines, which often results in spontaneous abortion. LF, a beneficial protein existing at high amounts in humans, has fewer side effects than expected.

Conclusion

These results show that rh-LF originating in mucus-producing cervical cells had both antibacterial and anti-cytokine actions. LF controlled inflammation and fetal survival in preterm delivery animal models. Thus, LF was clinically effective in preventing preterm delivery.

The present study examined the ability of rh-LF to prevent preterm delivery caused by bacterial infection. These findings support the clinical application of rh-LF in the prevention of preterm births via the suppression of inflammatory cytokine production.

References