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Lactoferrin and Necrotizing Enterocolitis in Preterm Infants

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Abstract

Neonatal necrotizing enterocolitis (NEC) is the most important neonatal problem in nursery which is associated with high neonatal mortality and morbidity. NEC also leads to various long term complications, including short bowel syndrome, systemic infection, eye problems, nutritional deficiency and neurodevelopmental impairments. Lactoferrin (LF) is a component of breast milk and multiple actions that includes antimicrobial, antiviral, anti-fungal, enhance immunity and various other actions. A few studies have been completed and a number of trails are going on for evaluation of efficacy and safety of LF in the prevention of NEC. In future, LF prophylaxis and therapy may have a significant impact in improving clinical outcomes of vulnerable premature infants. This review analyse the role of LF in prevention and reduction severity of NEC in preterm infants, with emphasis on mechanism of action, recent studies and current studies going on all over the world.

1. Introduction

Necrotizing enterocolitis (NEC) is an extremely severe gastrointestinal (GI) problem that occurs primarily among premature infants, with an incidence inversely proportionate to gestational age at birth [1, 2]. NEC is associated with high mortality with rate ranging between 20 and 30% [3]. NEC also leads to many long-term complications, including short bowel syndrome, infection, cholestasis, nutritional deficiency and neurodevelopmental impairments [4]. Many interventions has been used to prevent NEC which includes probiotics, prebiotics, antibiotics, lactoferrin (LF), Minimal enteral nutrition (MEN), restrict fluid management, slow increment in enteral feeds, preventing use of formula feeds and promoting breast milk usage in very low birth weight (VLBW) and extremely low birth weight (ELBW) neonates [5-7]. Many of these methods have proven with effect for prevention of NEC.

A lot of components in human milk may interact and provide a substantial benefit in preventing NEC, but the actual mechanisms remain unknown [8]. LF was identified as a whey protein in 1939, and was isolated and purified from bovine and human milk in 1960 [9, 10]. During the past fifty years, investigations have shown LF can improve the immune system development in the gastrointestinal tract of neonates. But gastrointestinal immaturity and feeding intolerance limits the benefits of human milk intake in extreme to very preterm infants. Some reports showed that early enteral administration of bovine LF (bLF) to VLBW infants or preterm infants can decrease the incidence of late-onset sepsis

and NEC [11-13]. The supplementation of infant formulas with appropriate levels of LF to prevent or reduce NEC in preterm infants should be more carefully studied. In this review we analyse the role of LF in prevention of NEC in preterm infants, with emphasis on mechanism of action and the advance in recent studies.

2. Mechanisms of LF Prevents NEC

LF is a 78kDa member of the transferrin family and present in body fluids, such as breast milk, saliva, tears, airway mucus and the secondary granules of neutrophils. LF may be the most important protein consumed by breast-fed infants after birth. Although the volume of milk consumed is low in the first 2~3 days, the content of LF in colostrum is high, up to 9g/L [14]. The content of LF in mature milk decreases to 2-3g/L over time, but the intake of LF remains elevated when the preterm infant continue receives breast milk [14]. However, GI immaturity and feeding intolerance limits the benefits of human milk intake in VLBW infants. Therefore, many investigators have started to overcome this problem by providing supplement LF after birth to prevent NEC [13].

2.1. Microbicidal Activity of LF

In the stomach, consuming LF can release lactoferricin (LFcin), which is responsible all the properties of LF. LFcin can kill a wide range of bacterial, fungal, viral, and parasitic pathogens [10, 15]. Breast milk contains LF and lysozyme which can act together in the stomach to destroy pathogens and eliminates damaging toxins on epithelia or mucosal invasion [15]. There was lower LFcin in the stomach of preterm infants than that in term infants because gastric pH is initially high and pepsin content is low in premature infants. The association of H₂ blockers with NEC suggests that insufficient production of LFcin when the gastric pH is elevated may be a key risk in increasing the incidence of NEC [16]. Lysozyme and other antimicrobial proteins are present in human milk. A combination of lysozyme and LF can cause the rapid destruction of *Escherichia coli* [17]. LF is particularly resistant to degrade in GI tract compared to other milk proteins like casein [18]. Thus, intact LF is still available in small bowel to act with lysozyme or other antimicrobial proteins such as defensins secreted by Paneth cells. Hence, a primary function of LF is in coordination with other antimicrobial agents in the neonatal stomach, thereby killing potential intra-gastric pathogens and allowing nearly sterile fluid to flow into the duodenum. LF can still block toxicity or invasion of epithelia by microorganisms. This mechanism avoids epithelia-related injury and involves LF binding to endotoxin, peptidoglycan, or Toll-like receptors [19, 20]. This consequence of LF prophylaxis is fundamental to decreasing the inflammation associated with NEC in preterm infants. LF may provide an initial level of protection via its glycan chains that contain sialic acid which bind proteins of viruses and bacteria that means pathogens can be carried out the body on LF and eliminated in the feces [6].

Reducing the lesion of virulent and invasive microbes and their toxic metabolites in the small intestine may be the main mechanism of LF in preventing NEC [6-19].

2.2. Intestinal Microbial Ecology and LF

Soon after birth, the neonatal intestine is confronted with a massive antigenic challenge of microbial colonization. Colonization by pathogenic bacteria and immature response to microbial stimuli can result in inflammatory response as seen in NEC. Commensal bacteria regulate intestinal innate and adaptive immunity and provide stimuli for ongoing repair and restitution of intestinal epithelial barrier, which appear to provide protection against intestinal inflammation and NEC. Many in vitro studies previously reported the growth-promoting effects of LF on bifidobacteria. It was called bifidogenic effect. The involvement of bound iron, sugar chains, and LFcin has been proposed in this bifidogenic mechanism [21]. The administration of LF-containing formula to infants increased bifidobacteria levels in the feces; however, human milk achieved better results than LF-containing formula [21]. A recent study in piglets found that feeding recombinant human LF (rhLF) significantly increased bifidobacteria in the ileum and lactobacilli throughout the small and large intestine [22]. It was also found that feeding Bifidobacterium bifidum could prevent NEC in a neonatal rat model and attenuate the expression of peptides in the small bowel. Elevated antimicrobial antimicrobial peptides are observed at the site of injury in animal models of NEC [23]. In conclusion, LF may act as a prebiotic to facilitate a gut microbiome that preventing NEC.

2.3. Immunomodulatory Effects of LF

A number of studies explained the influence of LF on T and B cell physiology. Prophylaxis supplemental given rhLF to neonatal rats significantly accelerated the development of Peyer's patches [24]. Akin et al [25] have conducted a randomized clinical trial in infants either VLBW or born before 32 weeks. Infants were assigned to two groups and receive either placebo or 200 mg LF daily during hospitalization. They found that lower incidence of sepsis were observed in LF-treated infants (4.4 vs. 17.3/1,000 patient days, p=0.007) and with none developing NEC, but without statistical significance. An increased T-regulatory (Treg) cells levels in LF group was observed. This suggested that LF prophylactic use reduced nosocomial sepsis and increase in Treg levels can be the mechanism for protective effects of LF on nosocomial sepsis [25]. LF also binds endotoxin, changing its inflammatory nature, as the LF-LPS complex can either prime or activate immune cells [26]. Thus, LF may activate an immune suppressed intestinal immune system in neonates that is a holdover of the fetal habitat. Mechanisms associated with rhLF-related maturation of dendritic cells have recently been described [27]. Data have shown that LF triggers the maturation of both human and mouse dendritic cells through TLR-2 and

TLR-4 [27].

2.4. LF Increases Intestinal Cell Growth

In mice, bLF supplementation can increase jejunal villus height and the expression of several intestinal brush border membrane enzymes. Addition of bLF or hLF to undifferentiated Caco-2 cells was able to increase cell proliferation [28]. In vivo, enteral bLF can stimulate crypt cell proliferation. The increased β-catenin expression indicates that Wnt signaling may in part mediate the stimulatory effect of bLF on intestinal cell proliferation [29]. Nguyen and colleagues [30] have used a preterm pig model to investigate the protective effects of enteral bLF against NEC development. NEC incidence and nutrient absorption were similar between the bLF-enriched formula groups and control group. Low doses bLF (0.1-1.0g/L) increased cell proliferation via extracellular signal-regulated kinase (ERK), limited IL-8 secretion and prevented NF- $\kappa\beta$ and hypoxia-inducible factor-1 α (HIF-1 α) activation, suggesting anti-inflammatory effects of bLF. In contrast, at a higher dose (10g/L), bLF exerted adverse effects by reducing cell proliferation, stimulating IL-8 release, inhibiting ERK activation and up-regulating NF- $\kappa\beta$ and HIF-1 α activation. It was suggested that supplementation of infant formulas with bLF should therefore be optimised carefully.

3. Animal Model of LF to Prevent NEC

Neonatal animal model trials have been done for evaluating the role of LF in preventing NEC. Feeding recombinant hLF to newborn rats before an artificial intestinal infection with E. coli showed significant reduction in bacteremia and death [24]. This finding was confirmed in another animal trial in which feeding recombinant hLF and Lactobacillus rhamnosus GG (LGG) had more beneficial effect than feeding LGG alone in reducing gut-related translocation after enteral infection with E. coli and hLF enhanced intestinal colonization with LGG [31]. In vivo study, Nguyen and colleagues [30] found that a bLF dose of 10g/L decreased IL-1β levels in proximal small intestine, but did not protect preterm pigs against NEC. At this dose, bLF tended to exacerbate NEC severity and decrease intestinal functions by reducing small-intestinal weight and increasing intestinal permeability in pigs that develop NEC. But in vitro, they found only lower doses of bLF may potentially be beneficial for preterm neonates to alleviate the mediators of inflammation. Further studies are necessary to investigate the effects of various bLF doses before concluding any protective effects of this protein in preterm neonates.

4. Clinical Trails

There are four RCTs have reported to assess the safety and effectiveness of oral LF in the prevention of sepsis and NEC in preterm neonates [13]. The results showed that oral LF supplementation can decrease late-onset sepsis in preterm infants (risk ratio (RR) 0.49, 95% confidence interval (CI) $0.32 \sim 0.73$). Two RCTs have shown that the incidence of stage

II or greater NEC was also decreased (RR 0.30, 95% CI 0.12~0.76) [25-32]. Two RCTs have shown that all-cause mortality was decreased (RR 0.30, 95% CI 0.12~0.75). Oral LF supplementation with probiotic decreased late-onset sepsis (RR 0.27, 95% CI 0.12 \sim 0.60) and stage \geq II NEC in one study (RR 0.04, 95% CI 0.00~0.62). Oral LF with or without probiotics decreased fungal sepsis but not chronic lung disease or length of hospital stay (from one study). There was no adverse effects of LF reported in four RCTs. Therefore, evidence of moderate to low quality suggests that oral LF prophylaxis with or without probiotics decreases late-onset sepsis and NEC stage II or greater in preterm infants without adverse effects. Ongoing trials are needed to provide more evidence in preterm infants. Clarifications regarding optimum dosing regimens, type of LF (human or bovine), and long-term outcomes are still needed.

5. Conclusions

LF has multiple effects on various antimicrobials and can be benefit for the field of neonatology. Up to now all the evidences show that the biological effects of LF include: enhanced antimicrobial activity, established a healthy intestinal microbiome, promoted intestinal growth and maturation, and enhanced development of the intestinal immune system. Its activities in the gut are the basis for the effect that NEC might be made less severe or prevented by feeding this protein. This is the key for preterm infants. A mother's colostrum fed soon after birth is the best alternative when a LF supplement is not available for preterm infants [33].

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