

Necrotizing Enterocolitis: An Update on the Benefits of Breast Milk

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Abstract: Necrotizing enterocolitis (NEC) is the leading cause of death for preterm infants resulting from gastrointestinal disease. This review will focus on several components of human breast milk that may be beneficial in the prevention and treatment of NEC. The severe pathological features of NEC include inflammation, mucosal ulceration and disruption of the intestinal barrier. Despite maximal neonatal intensive care, the incidence and mortality rate of the disease remains high. Administration of breast milk, as well as donor breast milk, to preterm infants has been shown to reduce the incidence of NEC. Beyond this, there is no disease specific treatment for NEC. The immunomodulatory and protective properties of human breast milk have been evaluated in search of key components that may be utilized for the effective prevention and treatment of NEC.

Keywords: Necrotizing enterocolitis, breast milk, polyunsaturated fatty acids, epidermal growth factor, probiotics.

INTRODUCTION

Necrotizing enterocolitis (NEC) may present as a devastating disease that ravages the intestines of a premature infant from an uncontrolled, exuberant immune response [1]. Breast milk is known to contain many natural immunotherapeutic elements that can attenuate the incidence of NEC [3-7]. In particular, we will focus our update on the role of basic components of breast milk and breast feeding: growth factors, lipids, immunoglobulins, and microbes (Table 1).

<1500g) [8-9, 12]. The mortality rate ranges from 15-30% of cases [8-12]. The disease is characterized by several pathologic features including inflammation, mucosal ulceration and disruption of the intestinal barrier, which rapidly progresses to necrotic bowel with sepsis and multi-system organ failure [8-12]. Currently, there is no effective disease-specific treatment for NEC [8-9]. An exaggerated immune response may be exacerbated by a number of possible stimuli such as bacterial colonization or formula feeding [8-13]. With an abbreviated period of in utero intestinal maturation,

Table 1: Key Components of Breast Milk in NEC

| | |
|-----------------------|---|
| Growth Factors | Epidermal Growth Factor (EGF) |
| | Heparin Binding Epidermal Growth Factor-like Growth Factor (HB-EGF) |
| Fatty Acids | Polyunsaturated Fatty Acids (PUFA: Omega-3, Omega -6 Fatty acids) |
| | Branched Chain Fatty Acids |
| Microbes (Probiotics) | Bifidobacterium sp. |
| | Lactobacillus sp. |

Despite continued advances in neonatal intensive care, NEC remains the leading cause of death of preterm infants suffering from gastrointestinal disease with an occurrence of 0.5- 5 affected infants for every 1000 live births in developed countries [8-11]. NEC can affect any portion of the gastrointestinal tract, but more frequently involves the ileum and proximal colon. The most significant associated risk factors include low gestational age and very low birth weight (VLBW

preterm infants are thought to be susceptible to NEC because of the immaturity of the intestinal epithelial cells, with altered cell surface receptors, such as toll like receptor 4 (TLR4) [13], decreased motility, altered mesenteric perfusion, blood flow to the gut, and perhaps decreased exposure to amniotic fluid [14-15]. Underdeveloped tight junctions can also increase permeability and decrease the protective quality of the bowel [8-13]. Hypoxic conditions as well as specific mediators such as the gram-negative bacterial endotoxin, lipopolysaccharide (LPS) have also been implicated in the onset of the disease [8-13] (Table 2).

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Table 2: NEC Influential Factors

| | |
|-------------------------------|-------------------------------------|
| Low Gestational Age | < 28 Weeks |
| Low birth Weight | < 1500g |
| Bacterial Colonization | Maternal or nosocomial |
| Enteral Feeding | Breast milk, Formula, or Donor Milk |
| Intestinal Ischemia - Hypoxia | congenital heart disease |

Breast milk contains several types of biologically active components, which serve to provide nutrients and aid in the development of the gastrointestinal tract in the newborn [3-7, 16]. Breast milk banking may be a necessity for premature infants that do not have the option of a fresh breast milk supply. To achieve this goal, advocates have established breast milk banks throughout the world. Despite banked human milk being three times more expensive than commercial infant formula, one estimate suggests savings of \$8,167 in intensive care costs for each child supplemented with human breast milk instead of commercial formulas, based on NEC risk reduction [17]. These findings are in line with earlier work identifying major economic advantages of banked human milk using three different calculation models [18]. On the other hand, a randomized control trial by Schanler *et al.* confirmed the need for an alternative source to the birth mother's milk, as only 27% of mothers were able to meet lactation requirements in the study. Of note, the study did not show any benefit from pasteurized donor milk over commercial formula with regard to the incidence of NEC [19]. These findings were later called into question by a Cochrane meta-analysis of five trials that showed a beneficial effect of donor breast milk over formula milk [20]. Additionally, there has been a significant amount of research to examine the effects of pasteurization and processing on the constituents of donor milk [21]. Further efforts will be required to evaluate the optimal processing, source, and funding of breast milk banking if it is to develop as a sustainable and important global initiative.

GROWTH FACTORS

Human milk contains several biologically active peptides or growth factors in significant concentrations. As mentioned earlier, the intestinal barrier is significantly disrupted in NEC. Gut barrier integrity requires a homeostatic balance in the mucous coat production, peristalsis, and secreted antimicrobial factors. Epidermal growth factor (EGF) and heparin

binding epidermal growth factor-like growth factor (HB-EGF-like growth factor) are the two best studied peptides in demonstrating prevention and attenuation of NEC [22]. Both initiate cellular signaling *via* the EGF family of receptor tyrosine kinases [23]. Highlighting the fundamental role of the EGF family for intestinal homeostasis, EGF deficiency results in early death due to hemorrhagic enteritis [24].

EGF is a polypeptide implicated in a variety of functions throughout the body in a variety of normal and pathologic conditions. Within the gut, EGF plays a role in intestinal development and influences protein synthesis, stimulates DNA synthesis, and promotes intestinal cell proliferation. EGF resists digestion in the gut and is both heat and acid-stable [25]. EGF is present in amniotic fluid and breast milk and found in especially high levels in breast milk from mothers with premature infants [26]. Administration of EGF enterally or *via* supplementation of formula has been demonstrated to decrease the incidence of NEC [27]. Subsequent investigations have focused on understanding the molecular mechanisms by which EGF reduces intestinal apoptosis (programmed cell death), increases mucin production by goblet cells and goblet cell density with a resulting improvement in the intestinal barrier function [28]. More recently, EGF has been demonstrated to reduce autophagy in the intestinal epithelium in a rodent model of NEC [29]. Since the first treatment of human NEC with EGF was reported in 1991, additional studies have found salivary EGF levels to be lower in infants with NEC [30-31]. Additional trials are required before introducing EGF supplementation as a therapeutic option for NEC.

HB-EGF-like growth factor belongs to the EGF family of growth factors and was first isolated from macrophages [32]. Subsequently, HB-EGF-like growth factor was identified in human amniotic fluid and breast milk and found to be protective in a rodent model of NEC [33]. This benefit appears to be mediated by a number of mechanisms, including increased enterocyte (intestinal absorptive cell) proliferation and migration [34], reducing intestinal apoptosis [33], and supporting

microvascular blood flow [35]. More recent studies of HB-EGF-like growth factor in experimental models have advanced the role of intestinal stem cells in reducing intestinal injury [35]. Mesenchymal stem cells can differentiate to replace injured cells and improve intestinal integrity [36]. In fact, mesenchymal stem cells may act synergistically with HB-EGF-like growth factor to prevent NEC by promoting engraftment of mesenchymal stem cells [37].

POLYUNSATURATED FATTY ACIDS

The importance of dietary PUFA has been studied extensively. In particular, omega-3 and omega-6 fatty acids have been extensively evaluated in a variety of inflammatory diseases [38, 39]. In pediatric nutrition, the potential beneficial effects of PUFA were first explored with regards to cognition and development in preterm and term infants [40, 41]. In 1998, a small double-blind randomized control trial sought to examine the effect of PUFA on strictly formula fed infants at high risk for NEC [42]. Controlling for the amount of total lipids, the treatment arm received PUFA in the form of egg phospholipids (omega-3:omega-6 ratio of 0.44:1) and showed a markedly decreased incidence of NEC. Of note, the treatment formula also contained a significantly higher amount of phosphatidylcholine than control, a possible confounding factor. Subsequently, a British group conducted a larger prospective multi-center double blinded randomized control trial evaluating the effect of PUFA on the neurodevelopment of preterm infants [43]. Development of NEC was examined as a secondary treatment outcome (omega-3:omega-6 ratio of 0.68:1) and was unchanged compared to the control group. Unaddressed by either of these studies was the optimal ratio of omega-3 to omega-6 and whether it may or may not have an effect on overall outcome, which remains an area of active debate [39]. Interestingly, this ratio differs in the breast milk of mothers, with diet likely playing an important role as comprehensively reviewed by Brenna *et al.* [44-45].

To determine which component of PUFA contributed to the protection from NEC, Lu *et al.* demonstrated that three different formulations of PUFA in a rodent model of NEC were equally effective in decreasing the incidence of NEC as compared to a PUFA-free formula [46]. One formulation included egg phospholipids, similar to the 1998 trial, supporting the importance of PUFA rather than a role for phosphatidylcholine [42]. The potential mechanisms by which PUFA may alter influence intestinal injury and

inflammation was reviewed by Caplan and Jilling [47]. The most clearly delineated mechanism of action is mediated by platelet activating factor (PAF). The implication of PAF in the pathophysiology of NEC is through its effects on gut mucosal permeability and inflammation [48]. PUFA has been shown to decrease PAF receptor synthesis [49], modulate TLR4 expression level in the gut [13], and block PAF induced epithelial cell apoptosis [50]. Several mechanisms, including but not limited to, the cellular interaction of PUFA that allows for its effect on the PAF receptor and TLR4 expression, are yet to be elucidated and more work is required to identify the optimal dosing and ratio of PUFA [39,46]. In addition to PUFA, the branched chain fatty acids, another fatty acid constituent of breast milk, remain an active area of investigation [51].

IMMUNOGLOBULINS

Both immunoglobulins IgA and IgG are secreted in breast milk and have been thought to help attenuate the incidence of inflammation in NEC. Oral administration of IgA and IgG has been shown to produce a protective effect on the gastrointestinal mucosa. IgA is known to play a functional role in mucosal immunity, while IgG is thought to protect the immune system of developing infants. Interestingly enough, there is no conclusive evidence to show that NEC was reduced with the oral administration of immunoglobulins [52, 53].

PROBIOTICS

The effect of breast milk on gut flora has been consistently reported through studies comparing the bacterial composition in the stool of breast fed versus formula fed infants [54, 55]. The difference could be due to the nutrient composition in breast milk which favors a particular bacterial flora [56, 57]. Moreover, the sterility of breast milk has been challenged by multiple studies, suggesting that the presence of bacterial species may likely contribute to the infant's gut flora [56, 58-60]. The composition of the gut microbial flora and its effect on the developing immune system remains an active area of research and cannot be over emphasized [61, 62].

Perhaps the most studied, with regards to their beneficial effects in NEC, are the *Bifidobacterium* sp. and *Lactobacillus* sp., both of which colonize the gut of breast fed infants at higher proportions than their formula fed counterparts [54-60, 63]. Capitalizing on this observation, multiple prospective randomized

clinical trials have shown a clear beneficial effect of different formulations of these bacterial species on the incidence of NEC and its related mortality in premature infants (<1500g), as reviewed by the Cochrane group [64] and later by Ganguli and Walker [63]. The beneficial effect of probiotics in NEC may be attributed to their modulation of intestinal inflammation [65], gut barrier function [65], mucin production [66], and decreased apoptosis [67], as shown by various experimental models [63, 65-67].

CONCLUSIONS

NEC remains a devastating disease without a treatment. Mothers of premature infants face many practical challenges as they seek to provide breast milk for their children, including the stress of a sick child, delayed onset of nursing, and inability to nurse for prolonged periods of time. For physicians, utilizing breast milk may challenge their ability to meet the elevated nutritional needs of a very premature infant. For society, there are challenges to establishing reliable access to breast milk for mothers that are unable to nurse. Despite these issues, breast milk offers a significant benefit to premature infants at risk for NEC and the hope for developing disease specific treatment in the future.

ABBREVIATIONS

| | | |
|--------|---|--|
| NEC | = | Necrotizing Enterocolitis |
| VLBW | = | Very Low Birth Weight |
| LPS | = | Lipopolysaccharide |
| EGF | = | Endothelial Growth Factor |
| HB-EGF | = | Heparin Binding Endothelial-like growth factor Growth Factor |
| PUFA | = | Polyunsaturated Fatty Acids |
| PAF | = | Platelet Activating Factor |
| TLR 4 | = | Toll-like Receptor 4 |

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