

Probiotics and synbiotics in necrotizing enterocolitis: where are we?

Abstract

Preterm neonates are susceptible to complications, including necrotizing enterocolitis, a leading cause of morbidity and mortality. Probiotics are live microorganisms, that when administered in adequate amounts, confer a health benefit to the host. Current evidence suggests that probiotics are effective in decreasing the incidence of NEC in preterm infants. Nondigestible oligosaccharides 'prebiotics' have a positive impact on the micro biota and represent another potential preventative therapy. The combination of probiotics and prebiotics is known as Synbiotics. Probiotics have numerous health benefits, and immune modulation and micro biota restoration are among the basic mechanisms leading to these benefits. Despite the widespread administration of probiotics to preterms in many countries, its adoption as a protocol in North America has been limited. The aim of this article is to review the current evidence and to update health care professionals on the use of probiotics and synbiotics in improving intestinal health, and in preventing NEC in preterm infants.

Keywords: necrotizing enterocolitis, probiotics, prebiotics, synbiotics

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Abbreviations: GA, gestational age, VLBW, very low birth weight, GI, gastro intestinal, NEC, necrotizing entero colitis, RCT, randomized controlled trial, CFU, colony forming units

Introduction

Premature or preterm infants are those born before 37 weeks' gestational age (GA).¹ Low birth weight is associated with prematurity and defined as birth weight <2500g. Premature infants, especially very low birth weight (VLBW), are at risk of neonatal morbidity and mortality. Immaturity of the organ systems of preterm infants makes them more susceptible to many complications including respiratory distress syndrome, bronchopulmonary dysplasia, necrotizing enterocolitis (NEC), patent ductus arteriosus, sepsis, anemia, retinopathy of prematurity and intraventricular hemorrhage.² To survive the extrauterine environment, preterm infants often require special care and extensive support in the neonatal intensive care unit. Despite advances in neonatal care and new therapies, these complications remain a concern.³ NEC, an acute inflammatory necrosis of the GI tract, is a common emergent complication of prematurity worldwide, with significant morbidity including neurodevelopmental impairment and a mortality rate reaching 30%.⁴ The incidence of NEC is inversely proportional to infant birth weight, and varies from 6% in North America to 28% in other parts of the world.⁵ The growing evidence of bacterial health is showing that supplementing preterm infants with probiotics "live microorganisms that have health benefits" reduces the risk of having NEC.^{6,7} The combination of probiotics and prebiotics is called synbiotics, which may be as important as probiotics in improving premature infant gut's health.^{8,9} Probiotics have been widely used in many countries in clinical practice; however, they are rarely given to premature infants in North America, which made it noteworthy to review the available evidence. Therefore, this article aim is to provide an overview about premature infant gut health focusing mainly on the current knowledge on the use of probiotics and synbiotics to improve intestinal health and prevent one of the devastating complications of prematurity necrotizing enterocolitis.

The preterm gastrointestinal system

Aside from its digestive and absorptive functions, the gastrointestinal (GI) tract is an essential immune organ and the largest defense barrier protecting the host from pathogens, toxins and subsequent inflammation while allowing commensal bacteria to grow. The GI tract begins to develop at four weeks' GA, facilitated by amniotic fluid, and continues to mature throughout childhood under the influence of dietary and environmental factors.¹⁰ The maturity of this system is directly proportional to GA. The preterm infant's gut is immature in multiple functions including motility, digestion, barrier defense function, intestinal permeability, immune defense and anti-inflammatory control.¹¹ The immaturity of these functions can lead to significant pathological symptoms and complications such as feeding intolerance due to dysmotility and bacterial translocation – a phenomenon in which bacteria cross the 'leaky gut' of the premature infant and spread into lymph and blood, causing sepsis and multiorgan failure.¹² In addition, the production of digestive enzymes, mucus and immunoglobulins is inadequate, which can allow pathogenic invasion and intestinal injury. Furthermore, preterm infants in the neonatal intensive care unit experience delayed initiation of enteral feeding and are exposed to common medications, such as antibiotics and H2 blockers, all of which cause intestinal atrophy and alter the defense barrier and immunity, allowing epithelial adherence and bacterial translocation.¹³

The micro biome is a complex ecosystem consisting of more than 1000 species of live bacteria that play major roles in nutrition and in the development of the immune system.¹⁴ The development of the neonatal micro biome begins with the exposure of the fetus to microbes in the amniotic fluid¹⁵ and continues to diversify depending on factors such as GA, mode of delivery, hospitalization, antibiotic use and type of feeding.¹⁶ Unlike the micro biome of the term infant, the preterm infant micro biome is less diverse and is predominated by *Staphylococcus* species, with *Bifido bacterium* species being less well represented.¹⁷ This is due to the fact that preterm infants are primarily treated with a course of broad spectrum antibiotics.¹⁸ Another cause of disequilibrium in the intestinal micro biota is

bacterial colonization from the intensive care environment.¹⁹ These changes in the composition of the micro biome of the preterm infant can further alter the development of epithelial barrier mechanisms and gut immune function. Accumulating evidence has shown that imbalances in intestinal micro biota may enhance certain acute diseases, such as neonatal sepsis and NEC, and may be involved in the initiation of chronic diseases such as type I diabetes, inflammatory bowel disease and obesity.²⁰⁻²²

Necrotizing enterocolitis

It has been hypothesized that NEC results from the interaction between prematurity and hypoxic ischemic events in the perinatal period, which include low Apgar score, enteral feeding, episodes of apnea and administration of Indomethacin.²³ Although the exact etiology of NEC is not well understood, pathogenesis is believed to be due to a multifactorial process that is related to one or more of the following:

- i. Hypoxic ischemic events
- ii. Immaturity and dysfunctionality of the GI tract (e.g. impaired peristalsis and disruption of tight junctions)
- iii. Altered micro biota and
- iv. Enteral feeding.²⁴

The net result of the interaction among these factors is the invasion of the intestinal wall by bacteria, followed by bacterial translocation and release of inflammatory mediators. Other factors that may contribute to the development of NEC include-

- i. Insufficient production of epidermal growth factor, an enzyme responsible for cell proliferation and differentiation
- ii. Additional stimulation of platelet-activating factor, a phospholipid inflammatory mediator and
- iii. Increased production of nitric oxide, a vasodilator and free radical molecule.^{25,26}

During hypoxic ischemic events, it has been postulated that blood is shunted from the bowel to vital organs, such as the brain, and the reperfusion of blood to the intestine provokes a pro-inflammatory cytokine cascade in the gut.²⁷ The release of pro-inflammatory cytokines further disrupts tight junctions, causing an increase in intestinal permeability and, therefore, bacterial translocation.^{4,27} Moreover, impaired peristalsis of the preterm intestine may allow more time for the carbohydrate from enteral feeding to serve as bacterial substrate, thereby leading to bacterial invasion of the intestinal wall and inflammation. Another important factor is the compromise of serum immunoglobulin A production, which eventually eases bacterial translocation.^{4,27} The use of antibiotics, in addition to the previously mentioned factors, facilitates the proliferation of pathogenic bacteria, which may then induce a hyper immune inflammatory response in the preterm infant intestine causing intestinal necrosis.²⁷ The use of breast milk and probiotics are potential preventive strategies to reduce the incidence of this devastating complication.²⁶

Discussion

Probiotics and synbiotics

Due to the increase in awareness of the role of the intestinal micro biota in the initiation of many diseases, the application of probiotics is emerging as a promising therapeutic strategy for a number of health conditions such as antibiotic-induced diarrhea,

infectious diarrhea, ulcerative colitis, atopic eczema and cow's milk allergy.²⁸ Probiotics are defined as "live microorganisms which when administered in adequate amount confer a health benefit on the host".⁶ The most commonly used microorganisms are *Lactobacillus*, *Bifido bacterium* and *Saccharomyces* species.⁶ There are various potential mechanisms by which probiotics improve GI health. Consumption of probiotics has been shown to maintain mucosal barrier integrity by reducing intestinal permeability, increasing mucus production, restoring tight junctions and preventing bacterial translocation.²⁹ Once probiotic microorganisms colonize, they promote further beneficial bacterial colonization by reducing intra luminal pH, producing toxic substances against pathogens and competing with pathogenic bacteria for binding sites.³⁰ Probiotics enhance innate intestinal immune defenses by augmenting serum immunoglobulin a mucosal responses, increasing the production of short-chain fatty acids and increasing leukocyte phagocytosis.³¹ Moreover, probiotics modulate intestinal inflammation through activation of toll-like receptor pathways.³² Toll-like receptors are transmembrane proteins that are expressed in host defense cells and enterocytes, which when triggered by probiotic bacteria, result in T-cell differentiation that further enhances cytokine production and anti-inflammatory profile, and decreases proinflammatory cytokine levels.³²

Prebiotics are another potential therapy for intestinal disease. Prebiotics are nondigestible dietary ingredient, usually polysaccharides and oligosaccharides that selectively promote the proliferation of 'beneficial bacteria' thus improving ecological balance and intestinal health. The beneficial bacteria, in turn, ferment prebiotics and produce short-chain fatty acids that provide colonocytes with fuel, facilitate mineral absorption, improve intestinal motility and inhibit the growth of pathogens by decreasing intestinal pH. Fructo-oligosaccharides, such as oligofructose and inulin, are examples of prebiotics. Combinations of probiotics and prebiotics are known as synbiotics.^{33,34} Several studies have shown the positive influence of prebiotics and synbiotics in a number of diseases including irritable bowel syndrome, inflammatory bowel disease, colon cancer, obesity, type II diabetes and hypercholesterolemia.³⁵⁻³⁷

Probiotics and synbiotics in premature infants

A growing body of evidence from clinical trials has shown that administration of common probiotics may reduce the incidence of NEC.³⁸⁻⁴¹ However, some studies have not shown such effects.^{42,43} For example, Dani et al.⁴³ conducted a multicentre randomized controlled trial (RCT) in which the probiotic *Lactobacillus* GG (6×10^9 colony-forming units.cfu) was administered once daily from first feed to discharge day (mean \pm SD duration 47.3 \pm 26.0 days) to 585 VLBW preterm Italian infants. Although the probiotics group tended to have less NEC, urinary tract infection and sepsis compared with the control group, the differences were not statistically significant (1.4% versus 2.8%; 3.4% versus 5.2%; and 4.7% versus 4.1%, respectively). In contrast, Lin et al.³⁹ found that the probiotic mixture of *Lactobacillus acidophilus* and *Bifidobacterium infantis* significantly reduced the severity and incidence of NEC in 367 VLBW infants in Taiwan (5% probiotics versus 12.8% control), however, there were some signals that probiotics may increase the risk of sepsis in infants weighing less than 750g. The variation among study results may be related to the differences in NEC incidence around the world because the incidence is lower in European countries compared with Asian countries.⁵

A recent meta-analysis included 16 RCTs analyzing the effectiveness of probiotics in the prevention of NEC in preterm infants.⁷ The included trials were variable with regard to GA, birth weight, probiotic species, dose and duration. There was evidence

of significant risk reduction for severe NEC and all-cause mortality using probiotic supplementation (relative risk 0.43.95% CI 0.33 to 0.56; relative risk 0.65.95% CI 0.52 to 0.81, respectively). It was concluded that enteral probiotic supplementation may prevent NEC, supporting the administration of probiotics to preterm infants. In contrast, a systematic review by Mihatsch et al.⁴⁴ evaluated 15 RCTs and found insufficient levels of evidence to support the routine use of probiotics. Nevertheless, the authors recommended that specific probiotic strains, such as *Lactobacillus acidophilus* and *Bifido bacterium infantis*, may be beneficial in reducing the incidence of NEC and need further investigation.⁴⁴

Regarding prebiotics, currently available studies have shown the positive effect of prebiotic-supplemented formula on different outcomes such as stool characteristics, stool pH, GI transit time, feeding tolerance, growth and fecal colonization;⁴⁵⁻⁴⁸ among these trials, the occurrence of NEC was reported by two,^{47,48} which showed statistically non-significant results. In a meta-analysis performed by Srinivasjois et al.⁴⁹ assessing the efficacy and safety of prebiotics in reducing the incidence of NEC in preterm infants, only two trials included this outcome and both reported that it did not occur over the duration of the trial.^{45,46} They showed that the stool colony counts of *Bifido bacterium* and *Lactobacilli* were significantly higher in the prebiotic-supplemented group (weighted mean difference = 0.53.95% CI 0.33 to 0.73 log cfu/g stool). Currently, there is only one RCT that has examined the effect of certain synbiotics in preterm infants.⁹ The trial showed statistically non-significant differences between the groups except for increased *Bifido bacterium* colonization of stool in one of the synbiotics groups (P=0.011). No differences among groups with regard to NEC, sepsis or adverse events were observed, which may have been due to small sample sizes.

Conclusion

Preventing NEC has been an elusive goal for decades. Based on previous literature, using probiotics to produce a healthier GI system in premature infants may have a role in the prevention of NEC. However, the current evidence seems to be not conclusive due to the use of single- or multiple-strain probiotics with doses ranging from 10⁵ to 10¹⁰ cfu, the inconsistent duration of treatment and the heterogeneity of probiotic formulations. Therefore, supplementing breast milk with probiotics in premature infants should not be yet adopted as routine clinical practice in neonatal intensive care units in North America. In the future, conducting a national multi-center trial may be a necessity to justify for any recommendation for routine probiotic use in North America as well as to investigate the effect of synbiotics and probiotics in improving GI health, and in preventing morbidity and mortality among preterm infants.

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Conflicts of interest

The authors declare that there are no conflicts of interest and shall disclose any potential conflicts of interest in the future.

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