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Understanding the Biologic Therapies of Probiotics, Prebiotics, and Synbiotics

Exploring Current Evidence for Use in Premature Infants for the Prevention of Necrotizing Enterocolitis

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ABSTRACT

Necrotizing enterocolitis remains a significant cause of morbidity and mortality in very low-birth-weight infants (<1500 g), with current preventive strategies unclear. Scientific evidence has recently emerged, suggesting that probiotics, prebiotics, and synbiotics may effectively and safely alter the premature intestinal microbiota, enhancing a deficient innate immune response and maturing the intestinal barrier to prevent necrotizing enterocolitis development. Currently, formal recommendations do not support routine use of these dietary supplementations for premature infants. Here, we examine how probiotic, prebiotic, and synbiotic preparations physiologically alter the underdeveloped intestinal microbial environment to potentially reduce necrotizing enterocolitis incidence and discuss current evidence that has examined safety and efficacy factors potentially supporting routine use among the premature infant population.

Key Words: microbiome, necrotizing enterocolitis, prebiotic, prematurity, probiotic, synbiotic

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Intestinal microbiota altering biologic therapies—known as probiotics, prebiotics, and synbiotics—have recently been the focus of intense research exploring their impact on necrotizing enterocolitis (NEC) reduction in very low-birth-weight (VLBW) infants (<1500 g). Because NEC is associated with significant mortality and morbidity,[1] it is imperative that we seek prevention methods, which currently are ill defined. Although published data regarding biologic therapy as a means to reduce NEC seem compelling, controversy exists related to specific dosing regimens, preparations, and combination effects for routine use in VLBW infants.[2] The purpose of this review was to discuss the current efficacy and safety of these biologic therapies among premature infants, examine how they may prevent NEC onset, and present an overview of current scientific evidence examining their effects.

THE HUMAN MICROBIOME AND PREMATURITY

Launched in 2007, the Human Microbiome Project initiated programs to examine the healthy body's microbial environments.[3] Studies found that a delicate balance between commensal and pathogenic bacteria supports normal function, immunology, and homeostasis in the healthy intestine, and disruption of this balance may lead to disease onset.[4] In adults, dietary changes have been shown to alter the gut microbiome and increase the risk for inflammatory bowel disease and ulcerative colitis.[5]

Preterm infants lack adequate intestinal commensal or "healthy" bacterial flora, which regulate natural defense systems by promoting sufficient maturation, inflammatory response, and homeostasis in the underdeveloped gut.[6] Often, this population is born by

cesarean delivery, a method of birth that usurps the first natural inoculation of gut bacteria an infant receives from passage through the vaginal canal.[7] There is evidence that the early use of antibiotics, a common practice with this population, further alters the colonization of the preterm gut with healthy microbiota.[8] Furthermore, preterm infants may experience enteral feeding delay, prolonging the introduction of commensal bacteria present in human milk. Therefore, the immature gastrointestinal system of the VLBW infant with a dysregulated innate immune response and imbalanced microbiome is vulnerable to potential injury.[4,9] Tenuous mucosal surfaces may be subjected to bacterial translocation if disrupted, leading to infection and NEC.[10] Data suggest that the decreased colonization of commensal bacteria in VLBW infants may be a critical factor for NEC development, and routine use of biologic therapies to promote commensal flora growth may effectively alter intestinal microbiota to prevent NEC onset.[11–13]

OVERVIEW OF BIOLOGIC THERAPIES

Probiotics are live microorganisms that augment natural intestinal defenses by regulating inflammatory response, cellular proliferation, and apoptosis.[6] Although many strains of commensal bacteria have been identified, the most common species in premature infants are *Bifidobacterium*, *Lactobacillus*, *and Bacteriodes.*[6,13] When ingested, probiotics regulate epithelial cell apoptosis, signal anti-inflammatory processes, stimulate cytoprotective genes, and decrease tissue permeability by improving tight junction gaps (see Figure 1).[6] Apoptosis (programmed cell death) and anti-inflammatory signaling are protective mechanisms when adequately upregulated in response to invading bacteria. These processes are necessary in the developing intestine for adequate cell proliferation and removal of cellular wastes while promoting adequate and healthy epithelial growth.[6,13] Tight junction gaps decrease interstitial permeability to maintain gut homeostasis. Probiotics supply the immature intestine deficient in "healthy" bacteria, promoting intestinal function, maturation, and defense against potential harmful pathogens.[12,14]

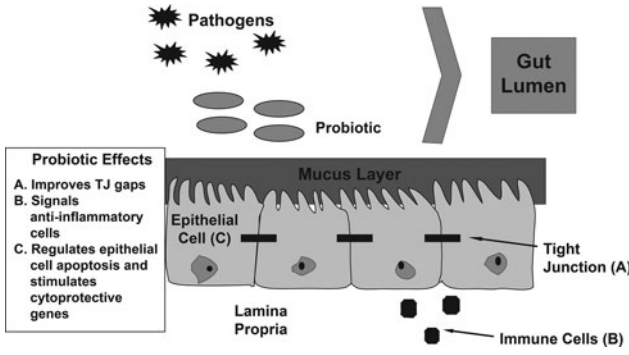

Figure 1. Probiotic mechanism of action at intestinal epithelial layer. The figure illustrates how probiotics augment natural intestinal defense by (A) strengthening tight junction gaps to prevent pathogenic bacteria from traversing epithelial barrier, (B) improving anti-inflammatory response through immune cell signaling, and (C) regulating epithelial cell apoptosis to remove wastes and stimulating innate cytoprotective genes.

Prebiotics are nondigestible fiber compounds that stimulate the activity and growth of healthy bacteria within the intestine.[15,16] They contain biologically active compounds that alter the interaction between pathogenic and commensal bacteria. Following ingestion, prebiotics remain undigested until they enter the colon. At this point, they undergo anaerobic fermentation that produces beneficial short-chain fatty acids (SCFAs) to naturally augment commensal bacterial activity and blunt the growth of pathogenic microbes by lowering gastric pH and promotion of adequate epithelial immunologic cellular function.[12] The most abundantly available prebiotics for infants are found in breast milk, known as human milk oligosaccharides (HMOs).[16] While HMOs serve to maintain intestinal microbial homeostasis for term infants, the immature function of the preterm intestine limits these processes. In addition, De Leoz et al[17] found the amount of HMOs in maternal breast milk highly variable between mothers delivering preterm and term infants and consistently immature HMO production throughout the time of breastfeeding in those delivering prematurely. Because breast milk provides natural immunity to premature infants, improves neurocognitive development, and improves gastrointestinal motility, dietary supplementation with prebiotics used in conjunction with breast milk may optimally augment intestinal innate immunity and microbiome stability in preterm infants.[16]

Synbiotics are a combination of both pre- and probiotics, which exert a synergistic effect. These therapies manipulate the intestinal microbiome by combining to compete with pathogenic microbes for binding sites on the cell surface.[12,15] Intestinal barrier function is enhanced through upregulated immune response to the introduction of active commensal bacteria (probiotic effect) and stimulation of commensal bacterial growth (prebiotic effect). Studies examining the use, efficacy, and safety of these preparations in preterm infants are scarce, warranting further scientific inquiry.

CURRENT EVIDENCE

The body of knowledge examining the therapeutic use and efficacy of biologic therapies to prevent or reduce

NEC onset is rapidly growing. Multiple recent studies of different designs, using varying types of probiotic dosages and feedings, suggest that routine use of biologic therapies—mainly probiotics—may decrease the risk for NEC development among VLBW infants (<1500 g). Both animal experiments and human clinical trials of differing evidential levels have explored potential mechanisms of action, specific strains most effective for prophylaxis, and indications for safe use among the preterm population. Major determinants for efficacy examined were decreased NEC incidence, late-onset sepsis, and mortality rates. Measures of safety included weight gain, stool consistency/changes, and feeding tolerance.

ANIMAL RESEARCH

Jakaitis and Denning[6] recently determined a possible mechanism of action related to intestinal microbiota alteration that mitigates an overexaggerated inflammatory response and matures the immune response in the underdeveloped intestine. Using premature intestinal epithelia in 0- to 3-week-old preweaned mice, investigators demonstrated that probiotic bacteria increased intestinal defenses by decreasing cellular apoptotic signaling, blocking inflammatory signaling, and maturing barrier function. Because unregulated apoptosis occurs during the early stages of NEC development, bacteria in probiotics, specifically *Lactobacillus rhamnosus* GG, can regulate intestinal epithelial apoptotic responses.[6] Also, using mouse models, it has been found that heat-killed probiotics (*Lactobacillus rhamnosus* GG) mature intestinal barrier function by promoting Claudin 3 expression, a protein that improves tight junction gaps.[13] Research using a different probiotic strain of *Bifidobacterium longum* subsp *infantis* in a rat model has also been described to significantly reduce the incidence of NEC and decrease the inflammation associated with disease onset.[18]

HUMAN RESEARCH TRIALS

A recently published large, multicenter, observational study conducted in Germany administered prophylactic daily dosing of Infloran, a *Lactobacillus acidophilus*/*Bifidobacterium infantis* probiotic. The incidence of surgical NEC was significantly reduced, and infants receiving probiotic prophylaxis had improved daily weight gain; however, no reduced incidence for sepsis was recognized.[19] Investigators from the ProPrem studies, a double-blinded, placebo-controlled, randomized trial using prophylaxis probiotic combination—*Bifidobacterium infantis*, *Streptococcus thermophilus*, and *Bifidobacterium lactis*—found that the occurrence of late-onset sepsis was unaffected with prophylactic probiotic use in infants born less than 32 weeks of gestation and weighting less than 1500 g.[20] In the same study, no incidence of modified Bell stage II NEC (presence of pneumatosis on abdominal radiograph)[21] or greater was reported in the probiotic group for 28 or more weeks of gestation, but the less than 28 weeks of gestation still had occurrence, although it was at a lower rate than that in the placebo group (11 vs 17).[20] In a retrospective cohort study, Hunter and colleagues[22] working with a probiotic containing *Lactobacillus reuteri* found that it significantly reduced NEC incidence in extremely low-birth-weight infants (<1000 g), with no significant differences in late-onset or gram-negative infections.

Several systematic reviews have analyzed recent data related to prophylactic probiotic use to prevent NEC (see Table 1). Most reports supported routine administration of probiotics as safe and efficacious in the preterm population[23–25]; however, there was expressed concern for long-term effects such as neurodevelopmental impact and growth patterns, factors that have yet to be longitudinally investigated.[26] Thirty-four studies were included with several overlap analyses between reviews, and most studies used different preparations of probiotics.[23–27] Therefore, interpretation of recommendations must be made cautiously. One study not included in these systematic reviews from China reported that prophylactic use of probiotics in VLBW infants was not associated with growth, neurodevelopmental delay, or sensory impairment in children 3 years of age.[28] However, questions remain regarding optimal preparation(s), dosing, and length of administration.[11] With these unknowns, clinicians may remain reluctant to adopt practice change.

Investigations examining the effectiveness of prebiotic use in preterm infants are less abundant. Prebiotic preparations added to the formula have been shown to have desirable effects related to weight, length, intake, stool characteristics, crying, regurgitation, vomiting, and fecal bacterial population counts in term infants.[29] Term infants exclusively formula-fed with prebiotic supplementation had similar weight/length gain, stool consistency, and less crying and feeding intolerance across a period of 4 weeks.[29] Investigators have examined the effects of this same prebiotic preparation on premature infants in a randomized controlled trial and found no facilitation in the reduction of intestinal permeability during the first week of life, thereby concluding that the risk for bacterial translocation and possible infection was not decreased.[30] These researchers also conducted a randomized controlled trial of 113 preterm infants treated with enteral supplementation of prebiotics (SCGOS/LCFOS/AOS) versus a placebo. They

Table 1. **Summary of investigations examining biologic agent use**[a]

Author	Year	Design	Findings
Probiotic studies			
Jakaitis and Denning[6]	2014	Animal	Probiotic bacteria reduce apoptotic signaling that blocks inflammatory signals and matures barrier function in immature intestinal epithelia
Underwood et al[16,18]	2014	Animal	Supplementation with probiotic *Bifidobacterium infantis* reduced NEC
Härtel et al[19]	2014	Observational multicenter comparison	*Lactobacillus acidophilus*/*Bifidobacterium infantis* probiotics reduced surgical NEC but not sepsis
Jacobs et al[20]	2013	RCT (ProPrem)	Probiotics *Bifidobacterium infantis*, *Streptococcus thermophilus*, and *Bifidobacterium lactis* significantly reduced NEC Bell stage II and greater but not sepsis
Hunter et al[22]	2012	Retrospective cohort	Probiotic *Lactobacillus reuteri* significantly reduced NEC but not sepsis or mortality
Wang et al[23]	2012	Meta-analysis of 20 RCTs	Probiotics significantly reduced incidence of NEC
Bernardo et al[24]	2013	Systematic review of 12 RCTs	Probiotics significantly reduced incidence of NEC Bell stage II and greater
AlFaleh and Anabrees[25]	2014	Meta-analysis of 24 RCTs	Probiotics significantly reduced incidence of NEC Bell stage II and greater
Mihatsch et al[26]	2012	Meta-analysis of 15 RCTs	Probiotic use cannot be recommended for VLBW infants due to inconsistency in dosing and preparations included in RCTs
Deshpande et al[8]	2010	Systematic review of 11 RCTs	Probiotic use showed 30% reduction in NEC incidence across trials
Deshpande et al[27]	2007	Systematic review of 7 RCTs	Risk of NEC was lower with probiotic use, sepsis rates did not differ, time to full enteral feeds was shorter
Chou et al[28]	2010	RCT	Growth and sensory outcome at the age of 3 y were not affected by probiotic supplementation given to VLBW infants
Braegger et al[40]	2011	Systematic review of 20 RCTs	Current evidence is inconclusive to support routine probiotic supplementation to reduce NEC
Prebiotic studies			
Veereman-Wauters et al[29]	2011	RCT	Prebiotic supplementation in formula-fed infants (SYN1 0.8 g/dL or GOS:FOS); stool consistency and bacterial composition were similar to breast-fed infants
Westerbeek et al[30]	2011	RCT	Prebiotic supplementation (SCGOS/LCFOS/AOS) does not enhance the postnatal decrease in intestinal permeability in preterm infants in the first week of life
Westerbeek et al[31]	2011	RCT	Prebiotic supplementation (SCGOS/LCFOS/AOS) does not affect fecal IL-8 and calprotectin levels
Srinivasjois et al[32]	2009	Meta-analysis of 7 RCTs	Supplementation with prebiotic oligosaccharides was not associated with decreased NEC incidence, late-onset sepsis, and time to full enteral feeds

(*continues*)

Table 1. **Summary of investigations examining biologic agent use[a] (*Continued*)**

Author	Year	Design	Findings
Synbiotic studies			
Dilli et al[33]	2013	RCT	Synbiotic supplementation (*Bifidobacterium lactis* plus inulin) decreased NEC incidence, sepsis, and death in infants ≥35 wk of gestation with CCHD
Underwood et al[34]	2009	RCT	Synbiotic supplementation increased *Bifidobacterium* stool content; no differences in short-chain fatty acid content, NEC incidence, or growth measures were observed

Abbreviations: AOS, acidic oligosaccharides; CCHD, cyanotic congenital heart disease; FOS, fructooligosaccharides; GOS, galactooligosaccharides; LCFOS, long-chain fructooligosaccharides; NEC, necrotizing enterocolitis; RCT, randomized controlled trial; SGOS, short-chain galactooligosaccharides; VLBW, very low-birth-weight.

[a]The reviewed research studies in this article that were conducted to understand and elucidate risks, benefits, and mechanisms of action of prebiotic, probiotic, and synbiotic as they relate to the preterm population.

examined that inflammatory markers of fecal IL-8 and calprotectin are elevated in preterm infants with NEC and hypothesized that intestinal inflammation would be reduced by the use of a prebiotic. Their findings revealed that this prebiotic preparation had no effect on these particular inflammatory markers, concluding no positive effect on the reduction of infection and morbidity.[31]

A recent systematic review analyzed results from 4 randomized controlled trials examining the efficacy and safety related to prebiotic supplements administered to preterm infants.[32] Findings revealed that prebiotics are well tolerated by preterm infants, demonstrated by improved gut motility, decreased pathogenic microbial colonization, and stool consistency similar to their breast-fed counterparts. Furthermore, no adverse effect on weight was seen in any of these studies, as weight gain was similar between those receiving prebiotic substances and those receiving only placebo. In 2 studies, no NEC was reported for infants given the prebiotic supplement, although these studies were not designed or powered to assess for effects of the prebiotic on the occurrence of NEC.[32]

Studies examining the effects of synbiotics—combination of prebiotic and probiotic preparations—in the preterm population are scarce. Researchers in Turkey conducted a randomized controlled trial with 100 infants, 35 or more weeks of gestation, who were born with cyanotic congenital heart disease. Half of the subjects received synbiotics (*Bifidobacterium lactis* plus inulin) and half received placebo.[33] The primary outcomes were NEC and nosocomial infections. The results revealed no incidence of NEC in the synbiotic group compared with 5 who developed NEC in the placebo group. Limitations to this study include a small sample ($n = 100$) and various types of cyanotic congenital heart disease. While the cardiac conditions varied, infants with obstruction of the right ventricular outflow tract were in the majority, with 51 of the 100 infants affected.[33]

Underwood and colleagues[34] found that synbiotic preparations (lactobacilli plus fructooligosaccharides, lactobacilli + fructooligosaccharides + bifidobacteria) versus placebo produced increased stool content of bifidobacteria in synbiotic-treated preterm infants; however, no differences in stool SCFA content, weight gain, or NEC incidence were evident among groups. Bifidobacteria, with anti-inflammatory properties, may be protective against NEC development and lactobacilli produce bacteriocins that kill pathogenic organisms.[35] It is hypothesized that biologic therapies containing these strains are optimal preparations for altering the premature microbiota providing protection against NEC. The presence of SCFAs in stool is thought to reflect colonic pH regulation and homeostasis, reducing the risk for acid fermentation and mucosal injury. Therefore, it remains unclear how these preparations affect overall colonic function and positively influence NEC rates.[33]

SAFETY ISSUES

Further research and understanding are needed to address issues of safety with these preparations. Recent reports from Switzerland detailing bacteremia in 5 very preterm infants receiving probiotic therapy using *Bifidobacterium longum* strains are very concerning.[36,37] Bacterial genome mapping on 2 of those cases found that the strain of bacteria grown

from infant blood cultures was the same strain as the probiotic.[36] Bacteremia from probiotics may occur for many reasons other than simple ingestion of live bacteria. Cross contamination can occur at several steps along the process of administration. For example, if a person uses the same workspace to prepare the probiotic and intravenous medications, live bacteria may be directly injected into the infant's bloodstream, potentially leading to bacteremia.

The biologic therapies discussed in this review are considered a food supplement and therefore are not regulated by the US Food & Drug Administration. Without this safety oversight, there is increased potential for contamination and/or disingenuous dosage advertisement. Safety remains a major concern for routine use of these products in the premature infant population. Manufacturer recalls, such as New Chapter, Inc's 2013 recall of its Probiotic Elderberry and Solgar, Inc's 2014 recall of ABC Dophilus Powder, have occurred because of safety issues with their product. In 2012, Sedona Labs issued a voluntary recall for iFlora Kids Multi-Probiotic Dietary Supplement due to possible *Salmonella* contamination.[38] The recall of ABC Dophilus Powder was a response to a very preterm infant who developed an intestinal mucormycosis following repeated administrations of the product and later died on October 11, 2014.[39] ABC Dophilus Powder was found to contain *Rhizopus oryzae*, which may cause mucormycosis.[39] Patel and Denning[13] noted in their research that the issues of safety and dosing have minimized the adoption of probiotics into routine clinical use in preterm infants. Current guidelines initiated by ESPGHAN Committee on Nutrition state that the administration of commercially prepared probiotic/prebiotic formulas is safe for healthy infants, although insufficient data exist to recommend routine use of these products. The committee further states that inadequate evidence precludes the safe use of these products for VLBW infants.[40] The American Academy of Pediatrics (AAP) statements concur with these recommendations, adding that evidence suggests that probiotics may prevent NEC in infants weighing more than 1000 g, but more research is needed.[41] Furthermore, there is no evidence that they will not prevent NEC in extremely low-birth-weight infants. The AAP in its 2010 Clinic Report on prebiotics and probiotic stated that "not all probiotics have been studied; therefore, all probiotics cannot be generally recommended."[41] It should be noted that many well-designed clinical trials have been conducted subsequent to implementation of these guidelines, yet many more are warranted. Therefore, continued vigilance and carefully designed large cohort clinical trials must be conducted for safety consensus guideline establishment.

CURRENT USE IN PREMATURE INFANTS

There is limited public data available that shows the prevalence of probiotics use among neonatal intensive care units (NICUs) in the United States, and no data are readily available examining the use of pre- or synbiotic use. In a presentation on behalf of the American Academy of Pediatrics Committee on Fetus and Newborn in 2014, 4% of NICUs within the Pediatrix Medical Group reported routine use of probiotic supplementation in 2012 for VLBW infants and 83% reported never using in any circumstance.[42] The Vermont Oxford Network reported that the use of probiotics in the same year was 8.3% in all gestational ages in data collection units and 5.7% for VLBW infants.[42] Only an estimated 4% of NICUs use probiotics across the United States for all gestational ages and 3.9% for VLBW infants. The committee also found that various preparations of probiotics were being used, and no general consensus on specific preparations was apparent.[42]

FUTURE DIRECTION

Recent evidence suggests that prophylactic use of probiotics may reduce the incidence of NEC in VLBW infants; however, further large cohort randomized clinical trials are warranted to justify recommendation and formal guideline change.[2,35] The prospect of synbiotic use seems appealing yet is grossly underexamined. Physiologically, theory suggests that the combination of pro- and prebiotics to synergistically alter the premature microbiota may be most beneficial. Because NEC continues to be a major cause of morbidity and mortality in VLBW infants, we must continue our quest in seeking effective, safe, and cost-saving preventative strategies without commercial market influence for selected preparations examined. Moreover, appropriate evidence defining optimal preparation and dosing guidelines for safe use among the premature population are necessary. Future research is essential to address current limitations of studies and provide guidance with regard to filtering through the heterogeneity of probiotic and synbiotic strains, identifying appropriate duration and timing of administration, along with the safest and optimum modes of preparation and delivery to the preterm population.

CONCLUSION

Data suggest that biologic agents alter the premature infants' developing intestine microbiota, improving function and inflammatory response. Here, we have presented numerous studies suggesting that probiotics reduce NEC incidence; however, studies supporting routine use of prebiotics and synbiotics remain

inconclusive. Because probiotics are live microorganisms and preparations are not federally regulated, controversy over routine use in the preterm population remains pervasive. Some believe that the high mortality rate associated with NEC in addition to available evidence supports practice change now while conducting ongoing clinical trials addressing knowledge gaps related to accurate dosing regimens.[43] However, providers will likely remain reluctant to adopt these changes until definitive data establish proper preparation and dosing guidelines for biologic therapy use in premature infants.

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