Early Human Development 85 (2009) S71–S74

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Probiotics and prevention of necrotizing enterocolitis

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ARTICLE INFO

Keywords:
Microbiota
Microbiome
Probiotics
Prebiotics intestine
Premature infant
Necrotizing enterocolitis

ABSTRACT

Necrotizing enterocolitis (NEC) is a devastating disease affecting primarily premature infants. Despite advances in neonatal care, the mortality rate following NEC has not changed significantly in the past 30 years. New preventative measures are needed. In this review, we will provide information to assess the possible role of probiotics, prebiotics and related agents in the prevention of this devastating disease. We will also discuss short and long term safety issues as well as potential alternatives. Although it is tempting to rebuild the intestinal microbiota using the agents such as pro and prebiotics during infancy, routine use is not yet warranted a cautious approach on the basis of sounds scientific data is needed.

1. Introduction

Necrotizing enterocolitis (NEC) is a devastating disease affecting primarily premature infants. Despite advances in neonatal care, the mortality rate following NEC has not changed significantly in the past 30 years [1]. There is significant short and long term morbidity associated with NEC including prolonged intravenous feeding requiring central line placement, and higher costs and longer lengths of hospital stay compared with gestational age-matched controls. Very disturbing recent evidence also documents significantly higher rates of neurodevelopmental impairment in patients with surgical NEC compared with birthweight controls [2]. The pathophysiology of this disease is multifactorial and consistent preventative measures have been elusive [3–6]. One very significant component of the pathophysiology of NEC in preterm infants pertains to the micro-ecology of the developing intestine. Recent reviews [7–10] tout the potential of live microbial agents, probiotics, in the prevention of NEC in premature infants. Along with enthusiasm, caution is being expressed in that the scientific basis as well as short and long term safety has not been established [11,12]. In this review, we will provide information to assess the possible role of probiotics in the prevention of this devastating disease. We will also discuss short and long term safety issues as well as potential alternatives.

2. Probiotics: evidence for prevention of NEC

The evidence for benefits of probiotics in this population is accumulating. In a multicenter double-blind study from Italy [13], preterm infants were randomized to receive either placebo or *Lactobacillus rhamnosus* GG, and the incidence of urinary tract infection, bacterial sepsis, and NEC were examined. Although there appeared to be a decrease in NEC in treated infants, this reduction was not statistically significant. Highly notable is the fact that the baseline prevalence of NEC in these centers was very low and a larger sample size would have been necessary to obtain adequate power in their study. In an open study from Bogota, Colombia, Hoyos [14] reported a reduction in the incidence of NEC in infants in a neonatal intensive care unit after the prophylactic administration of probiotic supplemented enteral feeding. The comparison was made with historical controls, the treating physicians were not blinded, and the study subjects generally had higher birth weights and were more mature (mean gestational age of 37 weeks, $<10\%$ of the babies being under 1500 g birth weight). Nevertheless, they reported an almost threefold decrease in cases of NEC and a fourfold decrease in NEC-related mortality. In a prospective, randomized blinded study in Taiwan, Lin et al. [15] reported a decrease in NEC and NEC plus mortality following probiotic prophylaxis. Another trial published from Israel found a reduced incidence of NEC from 14 to 1% in babies born weighing less than 1500 g [16]. These studies in total suggest a significant reduction in NEC with prophylactic probiotic supplementation, and recent meta-analyses confirmed these results [7,9]. The authors of one of these meta-analyses aptly emphasized the unanswered questions, which include the dose, duration, and type of probiotic agents (species, strain, single or combined, live or killed) used for supplementation as well as the assessment of long term effects [9]. Different probiotics were used in these studies. The most recently reported multicenter trial of probiotics from Taiwan [17] also suggests a beneficial effect against NEC with probiotics, but close scrutiny of the data also shows a higher incidence of sepsis in the smallest infants receiving probiotics, thus warranting significant caution.

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doi:10.1016/j.earlhumdev.2009.08.021

3. What are the mechanisms of probiotics?

The mechanisms of probiotic action appear to be multifactorial. In the distal small intestine and colon, probiotic bacteria in performing a "bioreactor function" [18] could enhance fermentation processes of the resident microbes that metabolize varying quantities of the short chain fatty acids (SCFAs) lactic, acetic, and butyric acids; synthesis of vitamins; and the production of antimicrobial bacteriocidins and fatty acids. In colonizing the intestinal tract, they also produce antimicrobial substances and modulate immune responses.

Their presence in the small intestine, primarily in the ileum can innate intestinal host defenses, including strengthening intestinal tight junctions, increasing mucous secretion, enhancing motility, and producing metabolic products (amino acids such as arginine and glutamine and short chain fatty acids) that secondarily function as protective nutrients. They contribute to the microflora diversity, thus helping to establish a normal commensal flora that protect against potential microbial pathogens by preventing the overgrowth of pathogens [18].

Intestinal epithelial cells, dendritic cells and other cell types possess special innate mechanisms that protect the intestinal mucosa, but may also result in overactive responses that could adversely affect the host. Toll-like receptor (TLR) signaling by the commensal intestinal microbiota is essential for homeostasis of the intestinal epithelium and protection from epithelial injury [19,20]. By recognizing pattern recognition molecules from commensal microorganisms, TLRs stimulate the production of epithelial repair factors. This is likely to be an important mechanism through which probiotics act. [34]. TLR activation by molecules such as lipopolysaccharide, flagellin, and lipoteichoic acid also generates the production of cytokines through intracellular signaling pathways, which activate transcription factors such as nuclear factor κB (NF-κB). Some nonpathogenic enteric bacteria have been shown to have an immunosuppressive effect on intestinal epithelial cells by directly inhibiting the NF-κB pathway. Others inhibit the same pathway by promoting the nuclear export of an NF-κB subunit, thus limiting the duration of NF-κB activation [18,21,22]. These inhibitory effects on the proinflammatory NF-κB pathway may be an important mechanism by which microbes regulate intestinal inflammation. Thus, the immunologic effects of probiotics are likely to occur through both less specific TLR-mediated actions on intestinal epithelial homeostasis and strain-specific effects on particular immune functions. Further work is needed to elucidate these details for specific probiotics in specific disorders.

4. What are the concerns about probiotic usage in premature infants?

One of the known mechanisms of probiotics is the capability to modulate an over-aggressive inflammatory response [21,23,24], but in some cases may actually incite an inflammatory response of their own in highly susceptible individuals [11, 25]. There is current concern that these live bacteria may translocate to the locally draining tissues and blood causing bacteremia, especially in immunocompromised individuals. Despite considerable enthusiasm for use of probiotics in premature infants for the prevention of necrotizing enterocolitis based on randomized controlled trials [15,16], data from of one of the most recent multicenter trials suggests that the smallest group of these infants may be at increased risk for sepsis when given prophylactic probiotics [17].

The promising short term effects of probiotics against NEC should intensify our desire to evaluate the long term effects of probiotics. Very little information exists on the long term microbial ecology of individuals exposed to probiotics in early infancy. This is especially critical because the organisms initially colonizing the gut at birth may establish chronic persistence in many children, in contrast to effective and prompt clearance if encountered in later infancy, childhood or adulthood [12]. One study by Rinne et al. [26] performed follow-up studies on a population who had been exposed to *L. rhamnosus* GG as infants. They studied stool samples taken at 6, 12, 18 and 24 months of age using fluorescent in situ hybridization and PCR. Numbers of different types of stools, vomits and crying time were comparable between the groups during the 7th and the 12th weeks of life. Dominant microbiota consisted of bifidobacteria throughout the study. At 6 months, there were less clostridia in feces in the placebo compared with the probiotic group ($P = 0.026$), whereas after long-term follow-up at 2 years, there were less lactobacilli/enterococci and clostridia in feces in the probiotic group than in the placebo group ($P = 0.011$ and $P = 0.032$, respectively), reflecting the impact of clostridia as a marker of microbiota succession in healthy infants. It is possible that with transient neonatal administration, probiotics induce some long-lasting effect (beneficial or detrimental) on the overall microbial ecology as well as the immune system, which persists beyond the disappearance of the actual organisms within the microbiota.

Several obstetric and perinatal factors have the potential to affect intestinal microbiota patterns in subsequent infancy. These include Caesarian versus vaginal delivery, use of antibiotics, mothers' milk versus formula and probiotics. The ramifications of early probiotic usage may be long term. Studies using non-culture based comprehensive analyses have just recently been initiated [27].

Similarly studies of long term health outcomes have just begun and are raising concerns. Contrary to some of the initial studies that demonstrated positive benefits of probiotics in patients with atopic dermatitis, considerable controversy is rising as studies are beginning to show that there may be detrimental effects [28]. For example, supplementation with *L. rhamnosus* GG (LGG) during pregnancy and early infancy neither reduced the incidence of atopic dermatitis nor altered the severity of atopic dermatitis in affected children but was associated with an increased rate of recurrent episodes of wheezing bronchitis [29]. There is also concern that they may form a persistent colony that prevents normal colonization of other microflora in the GI tract with subsequent alteration of the normal immune system development [30]. There is also concern that manipulation prior to establishment of a normal core microbiome in newborns may incur risks [12].

One 7-year follow-up study [31] where LGG was administered to mothers prior to delivery and then to the infants shortly after delivery showed decreased atopic dermatitis in the group receiving LGG. However, there were more cases of allergic rhinitis and asthma in the *Lactobacillus* GG group at 7 years of age. Although not statistically significant, the strong trend raises concern that attention needs to be given to long term health effects of early probiotic administration. More recent studies amplify the need for concern. One study from Germany observed neither a preventive effect of LGG on the development of atopic dermatitis nor any trend in this direction with LGG, but there was a statistically significant increase in wheezing bronchitis in the LGG-treated group [32]. Another study from Australia, points in the same direction regarding allergic sensitization, also suggesting that the routine use of probiotics for prevention of illness must be exercised with caution [33]. At the age of 12 months, the rate of sensitization to common allergens was significantly higher in the probiotic group [33]. Although the concern being raised in our proposed study in this application is focused on premature neonates, a formula for feeding term infants has been approved by the FDA and is currently on the US market without a systematic analysis of long term microecology using non-culture based techniques and overall health.

5. What are the alternatives: prebiotics, postbiotics, inactivated probiotics, microbial components?

These concerns prompt consideration of alternative agents such as prebiotics, which are usually indigestible oligosaccharides that

prompt growth of resident (hopefully beneficial) microorganisms. "Postbiotics", products of microbial fermentation, such as the short chain fatty acids acetate, propionate and butyrate may also provide beneficial effects [34]. However, determination of a specific dosage range is likely to be challenging since high doses of butyrate may actually damage the GI epithelium [35]. Accumulating evidence suggests that other specific components of microorganisms (usually those acting on toll-like and other signal transduction receptors in the intestinal epithelium, dendritic cells, and other immunoreactive intestinal cells) may confer the same benefits as probiotics without incurring the risks associated with a live organism.

6. Beneficial dead probiotics and their components: evidence for benefit?

Previous studies [34] demonstrated that live or heat-inactivated LGG are able to modulate tumor necrosis factor-α (TNFα) induced interleukin-8 (IL-8) production in the intestinal epithelium. The data suggest that although pretreatment with both forms of LGG was effective in down-regulating the TNFα inflammatory response, high doses of the live agent without pre-existing inflammatory mediator stimulation actually caused a large increase in the production of IL-8, whereas this was minimal with the heat-killed form. Thus, one might speculate that under certain conditions, the heat-killed form may be a safer alternative [32].

Because of the potential impact heat treatment has on cellular integrity and protein structure, additional studies are being done to determine whether alternative methods such as UV radiation can be used to inactivate probiotics and retain beneficial functions. The effects of live and UV-inactivated LGG on flagellin-induced IL-8 production in Caco-2 cells have also been evaluated [36]. One study showed a brisk induction of IL-8 production in the Caco-2 cells stimulated with flagellin that was blunted with pretreatment with both live and ultraviolet (UV) inactivated LGG [36].

The mechanisms of action of killed probiotic microbes as well as microbial components in intracellular regulation of inflammatory mediators are a topic of current investigation. Commensal microorganisms may be able to up-regulate the expression of intermediate "agonists" that regulate the production of inflammatory cyto- and chemokines [37].

7. Prebiotics as alternative option?

Prebiotics are defined as undigested nutrients that influence intestinal microbial flora [36]. The majority of commercially used prebiotic preparations use plant sources such as glucose oligosaccharide (GOS), fructose oligosaccharide (FOS) and inulin. Human milk contains prebiotic oligosaccharides (OSs) which promote the growth of beneficial gut flora including bifidobacteria and lactobacilli in newborn infants. Studies have shown that human milk oligosaccharides contribute 'bifidogenic' flora compared with formula-fed infants. Human milk OS has been shown to be beneficial in sepsis presumably by its action on the cytokine production, immunomodulation, and as receptor analog to inhibit the adhesion of pathogenic bacteria on the epithelial surface [40,41].

Some other studies in full-term infants (and one in preterm infants) have shown that oligosaccharide supplementation into formula influences gut colonization similar to breast milk feeding toward a more bifidogenic flora [37]. Furthermore, prebiotic supplementation has been shown to reduce atopic dermatitis and is associated with increased fecal IgA secretion in one particular report [38]. These findings suggest that prebiotics may work similarly to probiotics and that there could be a rationale for use in preterm infants for the prevention of NEC. Nonetheless, prebiotics for NEC prevention have yet to be studied [39].

One study suggests that prebiotic OS supplementation (GOS and/or FOS) is well tolerated by preterm infants and results in higher stool colony counts of bifidobacteria, reduced growth of pathogenic bacteria, accelerated GI transit time, softer and acidic stools similar to those of breastfed infants without adversely affecting the weight gain. It is very important to note that this review however related only to four articles [43–46] reporting mostly non-clinical outcomes [42] and did not show benefits in terms of prevention of diseases such as NEC or sepsis.

Additional carefully controlled studies are required before routine use of prebiotics in premature infants. This is especially important in the very preterm infant who is likely to also be sick and have a highly permeable intestinal barrier and may also be colonized with pathogenic microbes. One study in animals has shown increased translocation of intestinal microbes with the use of a prebiotic in preweaned rats being fed using the pup in the cup model [38]. It should also be noted that prebiotics may also promote the growth of resident pathogenic organisms that may be present in high quantities in sick low birthweight infants. Long-term follow-up is equally essential in such trials as survival without neurodevelopmental impairment is the preferred primary outcome for high-risk neonates. Overall, based on the available limited evidence, OS supplementation is safe but cannot be recommended as a routine in formula-fed preterm infants [42].

Another approach that may be beneficial involves use of probiotic microbes concerning the manipulation of its energy sources. Alterations in diet affect the composition and, more importantly, the collective metabolic output of the microbiota. Thus, deliberate dietary supplementation with bacterial fermentative substrates, usually complex carbohydrates can increase the growth of potentially beneficial microorganisms as well as the production of SCFAs, including butyrate, and indirectly modify immune function [47]. This approach is called "synbiotics." Obviously, the emerging ability to couple metagenomics of bacterial populations with resultant metabolomics will facilitate rational attempts to manipulate endogenous gut microbiota by dietary changes [48].

8. Conclusion

New information about the importance of normal establishment and maintenance of the intestinal ecosystem during the immediate neonatal period and early life is emerging. Perturbation in this ecosystem especially during early infancy may have consequences that extend well beyond the neonatal period and manifest as diseases in the later life. Although it is tempting to rebuild the intestinal microbiota using the agents such as pro and prebiotics during infancy, a cautious approach on the basis of sounds scientific data is warranted. The available evidence is therefore insufficient to derive any clear conclusions and does not support the routine supplementation of preterm formula with prebiotics and bacterial components.

Acknowledgement

This work was partly supported by the National Institute of Research Resources, National Institutes of Health; grant numbers: RO1 HD 059143 and M01RR00082 and an educational grant to M. Mshvildadze from the European Society for Pediatric Research.

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