Leading article

# Probiotics and necrotising enterocolitis in premature infants

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The intestinal ecosystem consists of three components that interact closely: the host cell, nutrients and microflora. Knowledge of the interactions among these components may be applicable for disease prevention.[1]

## INTESTINAL MICROFLORA

The microflora of adult humans are found primarily in the colon and distal small intestine, and consists of $>10^{13}$ microorganisms, comprising nearly 500 species.[1 2] The microflora exist in a mutually beneficial relationship with the host, are metabolically active, and allow for the synthesis and breakdown of numerous dietary compounds. Hence, the host does not need to adapt to perform these functions. In return, the intestinal bacteria are provided a protected, nutrient-rich environment. This mutual relationship may be important in the immature or neonatal intestine, because microbial digestion avoids the need for a mature enzyme capability. For example, a major nutritional effect of intestinal microflora is the metabolism of unabsorbed carbohydrates to short-chain fatty acids, an energy source for intestinal cells, and the production of vitamin K, the predominant source of this vitamin for the infant.

In a newborn, the intestine is colonised by 12–24 h. Infant diet determines the early content of the intestinal microflora.[3] Stools of breastfed infants have a predominance of *Bifidobacterium* and *Lactobacillus* species, which compete with *Bacteroides*, *Clostridia* and *Enterobacteriaceae* found as intestinal flora in formula-fed infants.[2] The bifidobacteria and lactobacilli ferment carbohydrates to produce lactic acid, creating an acidic intestinal milieu that favours the growth of non-pathogenic bacteria.[4] By contrast, the flora of formula-fed infants ferment carbohydrates to produce carbon dioxide and water, resulting in a neutral intestinal pH. The microflora also enhance intestinal barrier function to prevent bacteria from traversing through the intestine to extraintestinal sites: a process called bacterial translocation. The lactic acid bacteria do not translocate, and they produce bacteriocins that have antimicrobial functions and also prevent translocation of other bacterial species.[4]

## PROBIOTICS

Probiotics are defined as live non-pathogenic microbial preparations that colonise the intestine and provide benefit to the host.[4–6] An ideal probiotic agent must be healthy, resist degradation by acids and bile salts, adhere to intestinal epithelial cells, be considered non-pathogenic and non-invasive, modulate immune responses, be sensitive to usual antibiotics without the development of resistance, originate from human microflora and resist technological processing.[4] Probiotic microorganisms generally consist of strains of *Lactobacillus*, *Bifidobacterium* and *Streptococcus*. Bifidobacteria are part of the human microflora, but species differ according to age; newborns are colonised readily by *B breve* and *B infantis*, and colonisation is favoured in breastfed infants compared with formula-fed infants.[7] However, when given as a probiotic, bifidobacteria do not persist permanently in the intestinal tract when the dose is discontinued. Thus, it appears that each bacterial strain differs in its pattern of colonisation, clinical effects and dose needed to be functional.[5]

Probiotic agents, similar to the flora from which they originate, perform a myriad of functions, all to achieve an improved relationship with the host. They normalise intestinal microflora, increase mucosal barrier function, reduce intestinal permeability, enhance immune defences and improve enteral nutrition. Several of these functions lead to a reduction in bacterial translocation. Probiotics can improve enteral nutrition by aiding in intestinal maturation, synthesising nutrients otherwise not made by the body (eg, vitamin K), producing protective nutrients (arginine, glutamine, short-chain fatty acids) and improving mucosal integrity, leading to a reduction in the use of intravenous feeding, which is a major risk factor for infection. The use of probiotics to promote feeding tolerance has been shown to be effective in premature infants.[8]

## PREMATURE INFANTS

Several studies indicate that under a variety of circumstances, children receiving probiotics have reduced infectious morbidity.[9–11] Premature infants, however, represent a population particularly suitable for probiotic treatment. They have immature organ systems, are at high risk for infectious morbidity, experience delayed feeding, have a delayed establishment of intestinal flora because of the sterile environment of their incubator and the neonatal intensive care unit, and often are treated with broad-spectrum antibiotics and steroids.[1] Thus, the delay in intestinal colonisation of premature infants makes them more susceptible to pathogenic colonisation than term infants.

## NECROTISING ENTEROCOLITIS

Necrotising enterocolitis (NEC) is the most commonly occurring gastrointestinal emergency in preterm infants. Some reports estimate a >10% incidence among infants weighing <1500 g, with mortality approaching 30%.[1] Approximately 25% of survivors experience long-term sequelae.[12] The causes of this intestinal catastrophe are complex, but common factors associated with the disease are prematurity, immaturity of the intestinal tract (impaired motility, impaired barrier function), intestinal ischaemia, microbial colonisation with pathogenic organisms and enteral feeding. The premature infant may be exposed to many antibiotics, which alter intestinal microflora to facilitate colonisation by more pathogenic organisms. Certain changes in flora activate the inflammatory cascade, leading to high expressions of pro-inflammatory mediators. A combination of all these events culminates in the manifestations of NEC.

## PREMATURE INFANTS, PROBIOTICS AND PREVENTION OF NEC

Emerging evidence suggests that probiotics may have a role in the control or prevention of NEC by reducing intestinal colonisation with pathogenic organisms, reinforcing the intestinal barrier and alleviating intestinal inflammation. Functions such as promotion of fermentation to produce organic acids and production of antimicrobial bacteriocins and fatty acids add further theoretical support to their role in protection from NEC. Probiotics also affect innate intestinal host defences by strengthening tight junctions, increasing mucus secretion and enhancing intestinal motility. Lastly, their colonisation might reduce

**Abbreviation:** NEC, necrotising enterocolitis

**Table 1** Randomised trials on the effect of probiotics on necrotising enterocolitis in premature infants

| Study | Intervention n/N (%) | Control n/N (%) | Relative risk | Risk difference | No needed to treat |
|---|---|---|---|---|---|
| Jerusalem[15] | 1/72 (1.4) | 10/73 (13.7) | 0.10 | 0.12 | 8 |
| Taiwan[16] | 2/180 (1.1) | 10/187 (5.3) | 0.21 | 0.042 | 24 |
| Italy[14] | 4/295 (1.4) | 8/290 (2.7) | 0.52 | 0.013 | 77 |

the pro-inflammatory mediators responsible for the intestinal tissue damage.

## CLINICAL STUDIES IN PREMATURE INFANTS

Studies of probiotics in premature infants have focused on improvements in feeding tolerance and the prevention of NEC. Feeding tolerance was investigated in a randomised trial of 91 infants in Japan.[8] The average gestational age of the study population was 28 weeks and birth weight 1000 g. The probiotic group received $5\times10^9$ organisms/day of *B breve*, and had higher faecal colonisation with bifidobacteria than the placebo group (73% *v* 12%, respectively). The probiotic group was colonised slowly (73%, 82% and 92% at 2, 4 and 6 weeks after birth, respectively). The data suggested that better colonisation rates were observed in the more mature infants. Because the probiotic group had less feeding tolerance issues, they received more milk, which resulted in a better weight gain outcome over the 30-day study compared with the placebo group.[8]

In a neonatal nursery in Bogota, Columbia, over the course of 1 year, 1237 newborn infants were given daily doses of probiotics *L acidophilus* ($2\times10^8$ organisms/day) and *B infantis* ($2\times10^8$ organisms/day) throughout their hospital stay.[13] This study also enrolled all newborns; so the highest risk group for NEC, those with birth weights <1500 g, represented <10% of study infants. Nevertheless, the incidence of NEC was 3% during probiotic treatment, considerably less than historical controls in the previous year when 6.6% of 1282 infants developed NEC.[13] The mortality from NEC was more than halved (14 cases) during the year when infants received probiotics compared with historical controls from the previous year (35 cases).

An Italian multicentre, double-blind, randomised placebo-controlled trial of probiotic supplement *Lactobacillus GG*, $6\times10^9$ colony-forming units per day, was conducted in infants who were born at <33 weeks gestation or 1500 g birth weight.[14] The *Lactobacillus* supplement was given once daily from the onset of enteral feedings to hospital discharge—approximately 50 days. Overall, this study reported a low rate of infectious morbidity. When compared with placebo, the *Lactobacillus* supplement group had a lower, but not significant, rate of NEC.[14] The incidence of sepsis (4.4% *v* 3.8%) and urinary tract infection (3.4% *v* 5.8%) in the supplement versus placebo groups did not differ significantly.

An intensive randomised, double-blind, placebo-controlled trial of a triple probiotic mixture (*B infantis* $0.35\times10^9$ organisms/day, *B bifidus* $0.35\times10^9$ organisms/day and *S thermophilus* $0.35\times10^9$ organisms/day) was carried out in Jerusalem.[15] The supplement or placebo was given with the first feeding and continued until a postmenstrual age of 36 weeks was achieved. The study was powered to enable the detection of a change in the incidence of NEC from the prevailing 15% to 5%. The groups were adequately matched for birth weight and gestational age and feeding issues. A significant difference was found in the incidence of Bell Stage 2 or 3 NEC in the supplement versus control groups (1% *v* 14%; $p = 0.013$). In addition, the probiotic group had significantly less severe NEC. No significant difference was found in the incidence of sepsis (43% *v* 33%; $p = 0.28$) in the supplement versus control groups. The study reported a similar distribution of human milk feeding and feeding tolerance between groups.

The study in Taiwan evaluated 367 breastfed infants, born weighing <1500 g (average birth weight 1100 g and gestational age 28 weeks), in a randomised, placebo-controlled trial of two probiotic supplements, *L acidophilus* ($2\times10^8$ organisms/day) and *B infantis* ($2\times10^8$ organisms/day), given in breast milk (either mothers' own milk (70%) or donor human milk (30%)) twice daily during the hospital stay beginning at approximately 1 week of age.[16] There was a significant reduction in death or NEC (5% *v* 13%), NEC stage at diagnosis, NEC stage 2 or 3 (1.1% *v* 5.3%), sepsis (12% *v* 19%), sepsis or NEC (13% *v* 25%), and combined outcomes of NEC, sepsis or death (17% *v* 32%) in the supplement versus the placebo groups.[16]

The three randomised trials depicted in table 1 can be compared because the Breslow–Day test shows homogeneity of the relative risk assessments. The data were combined using the Mantel–Haenszel method. The weighted, pooled estimate of relative risk is 0.40, with a 95% confidence interval of 0.18 to 0.90, suggesting a beneficial effect of probiotic treatment on reducing the incidence of NEC. The weighted risk difference summarising the presented studies, 0.023, indicates that the number needed to treat to prevent one case of NEC is 43 infants.

## ARE WE READY FOR PROBIOTIC TREATMENT FOR PREMATURE INFANTS?

Probiotics may offer potential benefits for premature infants. We are still in the early stages of understanding the numerous interactions that occur between the intestinal microflora and luminal nutrients, and their interaction with the intestinal microenvironment over time. Nevertheless, probiotic treatment provides a promising strategy to prevent NEC in premature infants. Bell[17] described various strategies that have been proposed for the prevention of NEC in terms of absolute risk reduction and number of infants needed to treat to prevent one case of NEC (table 2). Among these strategies,

**Table 2** Proposed strategies for preventing necrotising enterocolitis

| Strategy | Absolute risk reduction | No needed to treat |
|---|---|---|
| Antenatal steroids | 0.019 | 54 |
| Delayed or slow feeding | Not efficacious | — |
| Enteral antibiotics | 0.089 | 11 |
| Enteral IgG and IgA | 0.066 | 15 |
| Enteral IgG | Not efficacious | — |
| Judicious fluid administration | 0.084 | 12 |
| Human milk feeding | 0.069 | 15 |
| Probiotics | | |
| *Lactobacillus* GG only | 0.013 | 77 |
| Infloran (2 organisms) | 0.042 | 24 |
| ACDophilus (3 organisms) | 0.12 | 8 |

Ig, immunoglobulin.
Adapted from Bell[17] and from Bin-Nun *et al*.[15]

the use of probiotics compares favourably with, if not superior to, many of the strategies listed.

As evaluated in the Taiwan study,[16] the role of combined strategies, such as the use of human milk and probiotics, has not been explored fully. Others have identified an additive effect of breast feeding and probiotics on gut immunity.[18]

The selection of the optimal probiotic mixture is not clear. It seems that double or triple probiotic strains provide the greatest protection. The dose and frequency of dosing need to be discussed. One problem with probiotic organisms is that they have variable rates of colonisation. For example, the rate of colonisation of lactobacillus when given as a probiotic is variable, varying from 60% to 80%.[5 8 19] The premature infant has lower rates, from 50% in the 1500–1999 g birth-weight group to 25% in the <1500 g birth-weight group.[20] In addition, it is not clear whether colonisation of the particular probiotic, at all or for a particular period of time, is necessary. It is not clear whether intestinal colonisation is the most important factor in predicting efficacy in the prevention of NEC. It has been shown that killed bacteria or their DNA may be as effective. Studies have shown that conditioned media from the probiotic may induce intestinal inflammatory responses in vitro.[21]

Although the premature infant may benefit considerably from probiotic treatment, their immunocompromised status, in conjunction with their chronic illnesses and the presence of indwelling catheters and foreign bodies, emphasises the caution that must be applied before a decision is made for routine treatment with probiotics. Systemic infection as a result of such probiotic treatment is a possibility.[22–24] Despite the case reports, no untoward effects have been acknowledged in the >1000 patients who participated in the randomised trials of probiotic treatment in premature infants. However, it is noteworthy that a marked reduction in sepsis in infants treated with probiotics was found in only one of the three randomised trials.[16]

Thus, the worth of probiotics may be realised with additional data on its long-term effects on immune and gastrointestinal functions and safety. Each strain must be evaluated at the proposed range of doses to identify minimal and optimal effects. To avoid concerns regarding the safety of feeding live bacteria to premature infant hosts, studies also should include comparisons of bacteria and bacterial extracts. Therefore, we are ready to conduct these studies so that probiotics or their derivatives can be used in this high-risk population.

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