Potential Role of Probiotics in the Prevention of Necrotizing Enterocolitis

Carlo Dani, Roberto Biadaioli, and Firmino F. Rubaltelli

Division of Neonatology, Careggi University Hospital, Florence, Italy

Necrotizing enterocolitis (NEC) is an acquired syndrome affecting the gastrointestinal tract, defined as “ischemic–inflammatory necrosis of the neonatal bowel.” It is one of the most severe pathologies that can affect preterm infants. NEC occurs more often in preterm and low-birthweight infants than in term infants (1). Its pathogenesis remains obscure, but it is generally considered to be a multifactorial disease.

Three conditions other than prematurity are considered to be the main risk factors for the development of NEC: intestinal ischemia, bacterial colonization of the neonatal intestine, and the availability of substrate, such as formula or human milk, to encourage bacterial growth. The mechanisms whereby these factors interact to induce an inflammatory cascade that leads to NEC are not clear. Increased serum levels of cytokines, such as interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), platelet-activating factor (PAF), and nitric oxide (NO) are reported in infants with NEC (2,3). In particular, it is hypothesized that an increased production of NO in enterocytes could promote their apoptosis in the intestinal villi (3).

Prematurity and enteral feeding are now believed to be the only primary risk factors for NEC development, with bowel ischemia playing a secondary role. Feeding is associated with the development of NEC in ~90% of affected infants (4).

ENTERAL FEEDING AND NECROTIZING ENTEROCOLITIS

Recent studies have proved that small volumes of nutrients introduced early into the gastrointestinal tract can promote gut morphologic and functional maturation, leading to an increase in mucosal thickness and villus height, increased plasma concentrations of gastrointestinal peptides, and better coordination of gastrointestinal motility (5). Moreover, the early presence of intraluminal nutrients encourages the development of normal bacterial colonization. Early-fed infants achieve full enteral feeding more rapidly than do late-fed infants, without an increase in occurrence of NEC (6).

The type of milk fed also is very important. It is well known that in formula-fed infants, the risk of NEC is higher than it is in infants fed a mixture of formula and breast
milk, and mixed-fed infants are at greater risk than are infants fed exclusively on breast milk (7). Confirming the protective effect of breast milk against NEC, this syndrome is less common in those countries where breast feeding is widely practiced. In Italy, for example, where >85% of all newborn infants are fed with human milk, the incidence of NEC is only 1.7% (8).

The way in which breast milk exerts its protective effect is not fully understood. However, it is known that breast milk contains various immunoprotective factors, which can be subdivided into the following three groups: antimicrobial factors (lactoferrin, oligosaccharides, and specific antibodies); antiinflammatory factors (protease antagonists, PAF acetylhydrolase, epithelial growth factors, antioxidant agents, soluble receptors for inflammatory cytokines, antiinflammatory cytokines); and other immunomodulating factors (cytokines, nucleotides, and so on) (9).

GUT COLONIZATION AND NECROTIZING ENTEROCOLITIS

Gut colonization plays an important although not definitive role in the pathogenesis of NEC (6). The type of infant feeding closely influences the development of intestinal flora. In breast-fed infants, gram-positive microorganisms—such as enterococci, bifidobacteria, and particularly lactobacilli—represent the predominant intestinal microflora; of the gram-negative bacilli, Escherichia coli is the most frequent, but it is present in relatively small numbers. In bottle-fed infants, there are fewer bifidobacteria and a relatively larger number of E. coli; moreover, bacteroides and other anaerobes are detected in larger numbers, as in the adult intestinal flora (10).

PROBIOTICS AND NECROTIZING ENTEROCOLITIS

NEC most often occurs in very low birthweight infants admitted to neonatal intensive care units (8). Bacterial colonization of the bowel in preterm infants in neonatal intensive care units may differ from that of healthy term infants: it may be delayed or deficient, and may be influenced by methods of neonatal care, such as treatment with antibiotics or nursing in incubators (11–13). This can favor the overgrowth of microorganisms such as Enterobacteriaceae and coagulase-negative staphylococci, which are the most common pathogens involved in nosocomial infection in neonatal units (14) and which also can predispose infants to the development of NEC (15).

It has been suggested that overgrowth of pathogens might be prevented by encouraging colonization of the bowel with nonpathogenic bacteria (probiotics) of species normally resident in the gut of preterm and term infants (16). Probiotics compete with other microbes for binding sites and substrates in the bowel and produce a wide range of antimicrobial substances such as bacteriocins, microcins, reuterin, hydrogen peroxide, and hydrogen ions (17–20). Lactobacillus GG, a strain of Lactobacillus casei that is generally found in breast-fed infants’ stools, has been found to be effective in the treatment (21–24) and prevention (25–27) of infantile diarrhea, indicating a role for this probiotic in stimulating and regulating the intestine’s host defense mechanisms (28). Moreover, it is known that 2 weeks of feeding with Lactobacillus GG is effective in colonizing the bowel of preterm infants (29) without adverse nutritional
effects (14). Thus it is possible that *Lactobacillus* GG supplementation could contribute to a decrease in the occurrence of NEC in preterm infants.

To evaluate this possibility, we have just concluded a double-blind multicenter prospective study in which we found that administration of *Lactobacillus* GG \( [6-10^9 \text{ colony-forming units (CFU)}] \) once a day until discharge, starting with the first feed, was effective in reducing the occurrence of NEC in preterm infants after 7 days of supplementation (unpublished data).

*Lactobacillus* GG could have contributed to the prevention of NEC in our population in several ways. It is known that lactobacilli can reduce the number of intestinal pathogens by competing for their adhesion sites. This hypothesis has been confirmed by *in vitro* studies, in which the protection of two models of differentiated epithelial cells (HT-29 and Caco-2) by lactobacilli was tested (30). It was found that the presence of *Lactobacillus* La1 inhibits the adherence to intestinal epithelial cells by *E. coli*, salmonella, and yersinia by \(~80\%\) (30). Second, probiotics can promote a non-specific immune defense mechanism—that is, phagocytosis by monocytes /macrophages and polymorphonuclear cells; these phagocytes, when activated, can ingest and destroy foreign bodies such as pathogenic bacteria. It has been shown in adults that 3 weeks of supplementation with *Lactobacillus* La1 enhances leukocyte phagocytic activity against *E. coli* (31). Whether these effects were due to cellular activation or to serum factors that increase phagocytic activity is unclear. Third, lactobacilli have been shown to enhance humoral immune responses. An increase in the number of IgA-secreting cells has been shown to follow the administration of *L. casei* to mice (32) and to children who received *Lactobacillus* GG during the acute phase of diarrhea (26). With a suckling rat model, the antigen-specific immune response was significantly enhanced in rats receiving *Lactobacillus* GG with cow’s milk compared with the response in a group given cow’s milk only, mainly through promoting the uptake of antigens in Peyer’s patches and enhancing local secretory immune responses (33). Finally, several strains of live lactobacilli have been shown to induce the release of the proinflammatory cytokines TNF-\(\alpha\) and IL-6, reflecting stimulation of nonspecific immunity (34). These immunostimulating and immunomodulating effects of probiotics could favor the development of intestinal flora with a low pathogenic potential and explain the decrease in NEC observed in our study.

In our study the occurrence of NEC was lower than that reported by the National Institute of Child Health and Development in the United States in 1991 (10% in very low birthweight infants) (35), but was similar to that reported in Italy (1.7%) (8). Nonetheless, we found that infants who received *Lactobacillus* GG were significantly less affected by NEC. This result confirms a recent study by Caplan *et al.* (36) in neonatal rats exposed to risk factors for the development of NEC (formula feeding, 200 kcal/kg/day, and asphyxia). These investigators found that probiotic supplementation (using bifidobacteria) was effective in causing bowel colonization and reduced the occurrence of NEC in the supplemented rats. Plasma endotoxin and intestinal phospholipase A\(_2\) expression was lower in bifidobacteria-treated neonatal rats than that in controls, supporting the role of bacterial translocation and activation of the inflammatory cascade in the pathophysiology of NEC.
In humans, Hoyos (37) recently showed that the daily administration of \textit{L. acidophilus} \((2.5 \times 10^7 \text{ CFU})\) and \textit{Bifidobacterium infantis} \((2.5 \times 10^7 \text{ CFU})\) was effective in decreasing NEC occurrence in newborn infants admitted to an intensive care unit. She treated 1,237 newborn infants over the course of 1 year and used 1,282 newborn infants admitted to hospital during the previous year as controls. She found that the frequency of NEC was lower \((p < 0.0002)\) in the probiotic-treated group \([35 (2.8\%) \text{ of } 1,237]\) than that in the control group \([85 (6.6\%) \text{ of } 1,282]\).

**CONCLUSIONS**

NEC is a multifactorial syndrome. Its prevention may be achievable through avoiding the known risk factors, which are as follows: prematurity, intestinal ischemia, abnormal bacterial colonization of neonatal intestine, and the intestinal availability of substrates, such as formula or human milk, to potentiate bacterial growth. The prevention of prematurity and intestinal ischemia, although desirable, is not always achievable. Conversely, we can chose to use protective enteral feeding, such as human milk, and we may be able to prevent intestinal overgrowth of pathogens by supplementing preterm infants with probiotics.

The study of Caplan in an animal model (36) and our own study and that of Hoyos (37) in preterm infants seem to confirm that probiotics may have a role in preventing NEC in preterm infants. These studies, although preliminary, may justify further evaluation of the clinical effects of probiotics in a larger population of infants.

**REFERENCES**

PROBIOTICS AND THE PREVENTION OF NECROTIZING ENTEROCOLITIS


**DISCUSSION**

*Dr. Moro:* It appears from your presentation that you did not find any difference related to the feeds the babies were receiving, which means that human milk was not protective. Were these babies receiving fresh human milk or pasteurized milk?

*Dr. Dani:* About 50% of the two groups received fresh breast milk from their mothers. The remainder received pasteurized breast milk from a donor mother.
Dr. Moro: Did you see any difference between fresh and pasteurized milk in relation to the rate of NEC?
Dr. Dani: No, there was no difference.
Dr. Moro: My second question is related to the timing of the first feed. You mentioned "some days after birth." What does that mean? Were you using a regimen of minimal enteral feeding or were you not giving milk at all for some days after birth?
Dr. Dani: In our unit we generally start giving small amounts of milk on the first day, but in some other centers that participated in the study, milk feeding started at 3 or 4 days of life.
Dr. Moro: So the regimens were different in the various centers.
Dr. Dani: There were small differences in the times of starting feeds between the various groups, but they were not statistically significant.
Dr. Moro: Did you find any difference in the rate of NEC according to the different feeding regimens?
Dr. Dani: No, we tried to perform that type of analysis, but the numbers of patients were too small.
Dr. Vigi: Do you have any information on the bacteria in the stools of the babies who were treated and not treated? Did you do any microbiologic evaluation of the different strains or differences between the control group and treated group?
Dr. Dani: This is an interesting question. When we planned this study, we thought we would perform stool cultures, but we had problems with the different methods of performing the stool cultures in the different centers. However, in a recent study in about 20 preterm infants, Peter et al. in Germany (1) found that there was no correlation between the isolation of specific bacteria in the stool and the occurrence of NEC; all the patients affected by NEC had pathogenic bacteria in the stool, but there were no specific correlations between the types or species of bacteria and the development of NEC.
Dr. Vigi: As you do not know precisely what the lactobacilli do under these circumstances, I think it would be helpful to know something about the environmental ecosystem in trying to understanding their mode of action.
Dr. Dani: Bacteria are certainly important, but they are not the only pathogenic mechanism in the development of NEC.
Dr. Alieit: I was intrigued why you took 7 days as starting point of your evaluation, and not 5 days or 8 days, for example.
Dr. Dani: Because we think that this is the minimal time required by Lactobacillus GG to exert its immunomodulating effect.
Dr. Marini: It has been shown that the prevention of respiratory distress syndrome (RDS) with antenatal glucocorticoids can also prevent NEC. Do you have any data on that?
Dr. Dani: As for other variables, the occurrence of antenatal steroid treatment was similar in the two groups, ~70% in each group.
Dr. Marini: I think the babies who are most at risk of developing NEC are those with intrauterine growth retardation and absent diastolic flow in the uterine artery during fetal life. Did you make a comparison between babies with severe intrauterine growth retardation and the others?
Dr. Dani: Yes, we did, but we found no difference between the groups.

REFERENCES