ORAL PROBIOTICS PREVENT NECROTIZING ENTEROCOLITIS IN VERY LOW BIRTH WEIGHT NEONATES

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Objective **To test the hypothesis that normalizing the intestinal flora by administration of prophylactic probiotics would provide a natural defense, thereby reducing both the incidence and severity of necrotizing enterocolitis (NEC) in preterm neonates.**

Study design **Neonates ≤1500 g birth weight were randomized to either receive a daily feeding supplementation with a probiotic mixture (*Bifidobacteria infantis*, *Streptococcus thermophilus*, and *Bifidobacteria bifidus*; Solgar, Israel) of 10^9 colony forming units (CFU)/day or to not receive feed supplements. NEC was graded according to Bell's criteria.**

Results **For 72 study and 73 control infants, respectively, birth weight (1152 ± 262 g vs 1111 ± 278 g), gestational age (30 ± 3 weeks vs 29 ± 4 weeks), and time to reach full feeds (14.6 ± 8.7 days vs 17.5 ± 13.6 days) were not different. The incidence of NEC was reduced in the study group (4% vs 16.4%; $P = .03$). NEC was less severe in the probiotic-supplemented infants (Bell's criteria 2.3 ± 0.5 vs 1.3 ± 0.5; $P = .005$). Three of 15 babies who developed NEC died, and all NEC-related deaths occurred in control infants.**

Conclusion **Probiotic supplementation reduced both the incidence and severity of NEC in our premature neonatal population.** ***(J Pediatr 2005;147:192-6)***

At birth, the neonatal intestinal tract is virtually sterile. As the human intestine is naturally exposed to a contaminated bacterial environment, the bowel becomes colonized quickly after birth with a variety of bacterial species.[1] Throughout life, the human intestinal tract continues to serve as host to a complex and dynamic society of nonpathological bacteria. In an attempt to maintain a healthy gut luminal milieu, the body develops an intricate symbiotic equilibrium between this bacterial environment and its own immune system—an equilibrium that results in the preferential colonization of the gastrointestinal tract by a variety of "favorable" gram-positive micro-organisms, most notably *Lactobacilli* and *Bifidobacteria*.

In contrast, the preterm newborn intestine tends to be colonized by different micro-organisms, predominantly coliforms, *enterococci* and *bacteroides* species. Even among very low birth weight infants receiving breast milk, Sakata et al[2] found that the *Bifidobacteria*, commonly found in the term newborn gut, were undetectable in the intestinal flora during the first 1 to 2 weeks after birth and did not predominate until after the third week of life. Similarly, Blakely et al[3] reported the appearance of *Bifidobacteria* only late in the third week of life in low birth weight neonates. Gewolb et al[4] observed that *Bifidobacteria* and *Lactobacilli* are found in the stools of <5% of extremely low birth weight infants within the first month of life. The combination of an increase in potentially pathogenic, micro-organisms together with a decrease in "normal flora" found in preterm neonates is one of the factors that render these infants at increased risk of developing necrotizing enterocolitis (NEC).[5-8]

CFU	Colony forming units	NPO	Nothing by mouth
NEC	Necrotizing enterocolitis		

See editorial, p 143, and related articles, p 186, and p 197.

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We hypothesized that "normalizing" the gut flora of preterm infants via the prophylactic administration of "beneficial" bacteria known as probiotics would either decrease the incidence of NEC or reduce its severity.

METHODS

Patient Population

Preterm neonates, <1500 g birth weight, who were admitted to the neonatal intensive care unit of the Shaare Zedek Medical Center between September 2001 and September 2004, and who began feeding on a weekday, were recruited for study on the day that they were to begin feeds. The study was approved by our institutional review board, and parental consent was obtained. The infants were prospectively and randomly assigned to one of two groups. The study group received their regular feeds plus a daily probiotic feeding supplement of ABC Dophilus (Solgar, division of Wyeth Consumer Healthcare, Bergen County, NJ) diluted in 3 mL of expressed mother's milk when available or in 3 mL of Similac Special Care formula (Abbott Laboratories, Abbott Park, IL) when mother's milk was unavailable. The control group received their regular feeds plus a daily supplement of 3 mL mother's milk (with nothing added) when available or a premature infant formula (with nothing added) when mother's milk was unavailable. Fresh suspensions of supplements were individually prepared by study staff who were not directly involved in routine patient care every other day for each study infant. The suspensions were prepared in personalized sterile bottles by suspending 0.5 teaspoon of probiotic powder in 3 cc of mother's milk or formula (as above). This amount of ABC Dophilus provided a total of 1.05×10^9 colony forming units (CFU)/day consisting of 0.35×10^9 CFU *Bifidobacteria infantis*, 0.35×10^9 CFU *Streptococcus thermophilus*, and 0.35×10^9 CFU *Bifidobacteria bifidus*. This supplementation did not change the physical appearance of the milk, and the bottles were labeled only with the patient's name and identification number—with no indication of study group assignment. At feeding time, the staff nurse assigned to any infant on study would remove the supplement from the refrigerator and administer it together with the regular feed. Daily supplements were continued until the infants reached 36 weeks postconceptual age. Attending physicians and nurses caring for the infants were blinded to the group assignments. The amount of feeding was advanced slowly if tolerated, and feeding was stopped if there was any sign of feeding intolerance (gastric residuals, abdominal distension, hemepositive stools). Infants received total parenteral nutrition until 100 mL/kg/day were supplied by the enteral route.

Infants were evaluated for purpose of the study at weekly intervals, at which time basic feeding—and any evidence of feeding intolerance such as diarrhea, abdominal distension, or vomiting—and growth data were recorded. Sepsis was diagnosed by the presence of clinical signs of sepsis confirmed by a positive blood culture.

Whenever a study infant was suspected to have NEC, the infant was evaluated by the attending neonatologist in conjunction with the pediatric radiologist. Clinical status and abdominal films were reviewed, and, if the diagnosis of NEC was established, the infant was assigned a score according to the Bell staging criteria.[9]

Based on an estimated reduction in the incidence of NEC from a pre-study incidence of 15% to 5% with treatment, with a power of 0.80 and an α of 0.05, we calculated that a sample size of 140 was required. Parametric data are presented as mean ± standard deviations. Continuous variables were compared by using Student's t test; χ^2 analysis was used to ascertain significant differences in categorical variables between groups. Significance was defined as $P < .05$. Relative risks and 95% confidence intervals were used to compare the additional risk reduction of probiotic treated neonates developing NEC with that of control subjects. Significance for relative risk determinations was defined as a 95% confidence interval that did not include the value 1. Repeated measures analysis of variance was used to compare continuous variables between groups and over time (Sigmastat statistical software, SPSS, Inc., Chicago, Ill).

Results were analyzed using a modified intention to treat analysis. As such, any infant who was withdrawn from the study during the course of the protocol (one whose parents withdrew consent and one who developed rotavirus diarrhea) was analyzed according to intention to treat. However, three infants who never began the protocol were excluded from the final analysis. In each of these cases the order for study solution administration was either inadvertently never written or was not noticed by the nurses, and thus these infants never received any study solution.

RESULTS

Of the 145 babies analyzed, 72 were randomized to the study group and 73 to the control group (Table I). No significant differences between the groups were detected. There were no differences in the incidence of culture-proven sepsis of any kind and no differences in the amount of time spent receiving antibiotic therapy.

General Feeding Data

Feeds were started on similar days of life in both groups, and full feeds (defined as 100 mL/kg/day) were reached at similar ages (Table II). The distribution of human milk-fed versus formula-fed infants was similar in the two groups, as were the total number of days that the babies were not fed enterally (nothing by mouth; NPO) from birth until 36 weeks postconceptional age. There were no differences in the incidence of any signs of feeding intolerance such as diarrhea, abdominal distension, or vomiting between the two groups. As the study progressed, we noted a trend toward improved total weight gain in the study group. By week 6 of the study, the cumulative weight gain was 691 ± 208 g versus 594 ± 239 g in the study versus control groups, respectively, although these differences were not significant.

Table I. Patient characteristics

	Study group (n = 72)	Control group (n = 73)	Significance
Gestational age (wk)	29.8 ± 2.6	29.3 ± 4.3	.40
Birth weight (g)	1152 ± 262	1111 ± 278	.36
Apgar score 1 min	7 ± 2	7 ± 2	.24
Apgar score 5 min	8 ± 1	8 ± 1	.69
Male:Female	44:28	37:36	.27
Caesarian section	56 (78%)	57 (78%)	.78
Small for gestational age	18 (25%)	11 (15%)	.20
Total episodes of nonstaph. sepsis	11	10	.97
Total episodes of sepsis	36	28	.21
Total number of patients with sepsis	31	24	.28
Total days on antibiotics	12.5 ± 10.9	14.9 ± 15.0	.27

Data presented as mean ± standard deviation wherever appropriate.

Table II. Feeding data

	Study group (n = 72)	Control group (n = 73)	Significance
Feeds started (day of life)	2.7 ± 2.3	2.6 ± 2.3	.79
Age reached full feeds (day of life)	14.6 ± 8.7	17.5 ± 13.6	.13
Age TPN stopped (day of life)	16.6 ± 9.3	18.6 ± 13.2	.29
Totally human milk fed Mixed/formula fed	42:12:18	47:9:17	.69
Total # days NPO (up to 36 weeks)	5.2 ± 6.0	4.8 ± 5.7	.68
% Time NPO	11 ± 13%	8 ± 10%	.12

Data presented as mean ± standard deviation wherever appropriate. *TPN,* total parenteral nutrition.

Necrotizing Enterocolitis

The incidence of NEC in the control group was 16.4%, which was similar to the baseline incidence in the years before the current study. There were fewer cases of NEC in the study group than in the control group (3/72 [4%] vs 12/73 [16.4%]; relative risk 0.25; 95% CI: 0.075-0.86). Reducing the incidence of NEC from 17% to 4% yields a relative risk reduction of 75% and an absolute risk reduction of 12%, which means that eight infants would need to be treated in order to prevent one case of NEC. The incidence of clinically significant NEC (Bell Stage 2 or 3) was 1 of 72 (1%) versus 10 of 73 (14%), $P = .013$, in the study and control groups, respectively. All three of the most severe (Stage 3) cases occurred in the control group. Although there were no significant differences in the initial presentation of NEC between the two groups, those in the study group who did develop NEC had less severe disease, based on both Bell's criteria and mortality (Table III). Three of the 15 infants with NEC died; all three of the NEC-associated deaths were from the control group.

Mortality Data

Although the difference was not significant, it is of note that three infants in study group versus eight infants in the control group died ($P = .218$; RR 0.38; yielding a relative risk reduction of 62%; 95% CI: 0.38-1.38). As noted above, three deaths in the control group were attributed to NEC, whereas there were no NEC-associated deaths among the treated infants. There were no other significant differences in the characteristics of those who died between the two groups.

NEC and/or Mortality

When the combined incidence of NEC and/or mortality was compared, significantly more infants in the control group (17/73) were affected as compared with the study group (6/72) ($P = .025$; RR = 0.358; 95% CI = 0.150-0.856).

DISCUSSION

Probiotics may protect against NEC by shifting the intestinal ecological balance from a potentially harmful microflora to one that would be predominantly beneficial to the host.[10] The two principal kinds of probiotic bacteria are members of the genera *Lactobacillus* and *Bifidobacterium,* which predominate in the normal gut flora of healthy, breastfed, term neonates. Furthermore, probiotics offer protection by strengthening the intestinal mucosal barrier function, which, in turn, impedes the translocation of pathogenic bacteria.[11-13] Additional mechanisms also may contribute to the reduction in NEC following probiotic supplementation. In IL-10 knockout mice, probiotics decreased cytokine production both systemically and at the mucosal surface.[14] Other studies have implicated small proteins called bacteriocins, which are secreted by bacteria and are capable of killing other bacteria as the protective factor in probiotic therapy.[1]

Probiotic supplementation has resulted in a reduction in the incidence of NEC-like intestinal lesions in several animal models. Caplan et al[15] showed that *Bifidobacteria* supplementation resulted in intestinal colonization and subsequent reduction in NEC-like lesions in a neonatal rat model of intestinal ischemia/reperfusion. Butel et al[16] demonstrated, in a NEC model in quail, that supplementation with

Table III. NEC and mortality

	Study group (n = 72)	Control group (n = 73)	Significance
# cases NEC	3 (4%)	12 (16.4%)	P = .03*
BW of NEC infants (g)	949 ± 223	956 ± 223	P = .85
GA of NEC infants	26.8 ± 1.6	27.6 ± 1.9	P = .52
Apgar 1 min	4 ± 1	6 ± 2	P = .08
Apgar 5 min	7 ± 2	8 ± 1	P = .58
Age of diagnosis (d)	21 ± 9	21 ± 14	P = 1.00
Bell staging	1.33 ± 0.46	2.33 ± 0.46	P = .005*
NEC-associated mortality	0/3	3/12	P = .87
NEC and/or death	6/73	17/72	P = .025*

Data presented as mean ± standard deviation wherever appropriate.
BW, birth weight; *GA,* gestational age.
*Comparison of the given value in the study group versus the control group.

Bifidobacteria prevented the development of cecal lesions reminiscent of NEC.

Several studies of probiotic administration to premature infants have been published. One randomized controlled trial found that infants whose feedings were supplemented with *Bifidobacterium breve* had higher rates of fecal bifidobacterial colonization at 2 weeks of age (73% vs 12%), decreased gastric aspirate volume, improved weight gain, and improved feeding tolerance. However, the incidence and severity of NEC were not reported.[17] In a multicenter double-blind study from Italy,[18] preterm infants were randomized to receive either placebo or *Lactobacillus rhamnosus* GG, and the incidence of urinary tract infection, bacterial sepsis, and NEC was examined. Although there appeared to be a decrease in NEC in treated infants, this reduction was not statistically significant. The use of a single probiotic agent rather than two agents may explain, at least in part, the smaller treatment effect in this study as compared with other studies. However, the number of babies in this study who developed any of the three stated outcomes was surprisingly low (2.7%). In an open study from South America, Hoyos[19] 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. However, 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, Lin et al[20] reported a decrease in NEC and NEC plus mortality following probiotic prophylaxis. In this study, the untreated event rate also was lower than ours (5% vs 17%).

Given that the incidence of NEC can be quite variable across different institutions and even within the same institution at different times, randomized trials from different centers with different untreated event rates are important.[21] Despite differences in underlying event rates of NEC, our degree of relative risk reduction was similar to that of Lin's study (75% vs 79%, respectively). Furthermore, we found that not only the incidence but also the severity of NEC was reduced by probiotics. In fact none of our probiotic-treated infants died from NEC. Pooling the results of the three randomized trials, ours, Lin et al,[20] and Dani et al,[18] the overall relative risk reduction for NEC with administration of probiotics is 70%; the absolute risk is reduced by 4%, yielding 26 as the number needed to treat in order to prevent one case of NEC. Lin et al recommended combining NEC plus death in their analysis because death precludes the subsequent development of NEC and, as such, "prevents" NEC. When our results were analyzed in this way, we found a relative rate reduction for NEC or death of 64% and an absolute rate reduction of 15%, yielding as seven the number needed to treat to prevent one case of either NEC or death.

There is no clear indication from the literature as to the optimal strain of probiotic bacteria that should be administered to premature babies. The multitude of reported clinical studies to date have utilized different strains of probiotics and different administration regimens (dosage, length of treatment). Choosing which probiotics to use, therefore, was a challenge. Because *Bifidobacteria* form the greater part of the intestinal flora of breastfed infants, we felt it important to include *Bifidobacteria* in our regimen. We added *S thermophilus* in view of the fact that studies in other situations have demonstrated that supplementing *Bifidobacteria* with *S thermophilus* improved the efficacy of the supplement in preventing diarrhea secondary to rotavirus[22] and produced better long-term growth.[23]

Lactobacilli and *Bifidobacteria* are generally regarded as nonpathogenic because they naturally inhabit the intestine. Although isolated cases of *Lactobacillus* bacteremia have been described, these are rare, and seem to occur in immunocompromised or extremely sick babies receiving high doses of *Lactobacillus.*[24] It has even been argued that for bacteremia originating from endogenous flora (such as those occurring with NEC), infection with *Lactobacilli* is preferable to sepsis from other potential intestinal pathogens such as *Klebsiella*, *Enterobacter*, or yeast.[21] Furthermore, in the three previous clinical studies of probiotic administration to premature infants,[18-20] there were no adverse effects, including no cases of pathogenic infection caused by a probiotic organism. We observed no cases of sepsis (culture positive with any of the strains administered in the study) or other toxicity, such as diarrhea, reduced feeding tolerance, or increased susceptibility to infections, attributable to probiotic administration.

The pathogenesis of NEC remains elusive, the etiology multifactorial, and the pathophysiology incompletely defined. Four key risk factors have been identified—prematurity, formula feeding, intestinal ischemia, and bacterial colonization—as presumptive prerequisites to the initiation of intestinal injury in neonates. These risk factors, in turn, appear to stimulate activation of the inflammatory cascade

that ultimately results in the final common pathway of bowel necrosis.

In our opinion, the appeal of probiotics in neonatology is threefold. First, its safety record renders it an attractive alternative to many of the more aggressive therapeutic options; second, it represents a simple, non-invasive attempt to recreate a natural or normal flora rather than a disruption of nature; and third, it appears to be effective in preventing a major source of morbidity in low birth weight infants. Further studies must be performed to identify the ideal strain(s) and optimal dose and length of treatment required to prevent NEC. By demonstrating a probiotic-mediated reduction in both the incidence and the severity of NEC in premature infants with no accompanying adverse effects, our results lend further support to the consideration of inclusion of probiotics in the therapeutic armamentarium of neonatology.

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REFERENCES

1. Guarner F, Malagelada JR. Gut flora in health and disease. Lancet 2003; 361:512-9.

2. Sakata H, Yoshioka H, Fujita K. Development of the intestinal flora in very low birthweight infants compared to normal full-term newborns. Eur J Ped 1985;114:186-90.

3. Blakey JL, Lubitz L, Barnes GL, Bishop RF, Campbell NT, Gillam GL. Development of gut colonisation in pre-term neonates. J Med Microbiol 1982;15:519-29.

4. Gewolb IH, Schwalbe RS, Taciak VL, Harrison TS, Panigrahi P. Stool microflora in extremely low birthweight infants. Arch Dis Child Fetal Neonatal 1999;80:F167-73.

5. Claude EC, Walker WA. Hypothesis: inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. FASEB J 2001;15:1398-403.

6. Hoy C, Millar MR, MacKay P, Goodwin PGR, Langdale V, Levene MI. Quantitative changes in faecal microflora preceding necrotising enterocolitis in premature neonates. Arch Dis Child 1990;65:1057-9.

7. Lawrence G, Bates J, Gaul A. Pathogenesis of neonatal necrotising enterocolitis. Lancet 1982;1:137-9.

8. Hall MA, Cole CB, Smith SL, Fuller R, Rolles CJ. Factors influencing the presence of fecal lactobacilli in early infancy. *Arch Dis Child* 1990;65: 185-8.

9. Bell MJ, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, et al. Neonatal necrotizing enterocolitis: therapeutic decisions based upon clinical staging. Ann Surg 1978;187:1-7.

10. Fuller R. Probiotics in man and animals. J Appl Bacteriol 1989;66: 365-78.

11. Duffy LC. Interactions mediating bacterial translocation in the immature intestine. J Nutriion 2000;130:432S-6S.

12. Orrhage K, Nord CE. Factors controlling the bacterial colonization of the intestine in breast-fed infants. Acta Paediatr 1999;80:S47-57.

13. Mattar AF, Drongowski RA, Coran AG, Harmon CM. Effect of probiotics on bacterial translocation in vitro. Pediatr Surg Int 2001;17: 265-8.

14. McCarthy J, O'Mahony L, O'Callaghan L, Sheil B, Vaughan EE, Fitzsimons N, et al. Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. Gut 2003;52:975-80.

15. Caplan MS, Miller-Catchpole R, Kaup S, Russell T, Lickerman M, Amer M, et al. Bifidobacterial supplementation reduces the incidence of necrotizing enterocolitis in a neonatal rat model. Gastroenterology 1999; 117:577-83.

16. Butel MJ, Waligora-Dupriet AJ, Szylit O. Oligofructose and experimental model of neonatal necrotising enterocolitis. Br J Nutr 2002; 87(suppl 2):S213-9.

17. Kitajima H, Sumida Y, Tanaka R, Yuki N, Takayama H, Fujimura M. Early administration of *Bifidobacterium breve* to preterm infants: randomised controlled trial. Arch Dis Child Fetal Neonatal Ed 1997;76:F101-7.

18. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli F. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. Biol Neonate 2002;82:103-8.

19. Hoyos AB. Reduced incidence of necrotizing enterocolitis associated with enteral administration of *Lactobacillus acidophilus* and *Bifidobacterium infantis* to neonates in an intensive care unit. Int J Infect Dis 1999;3:197-202.

20. Lin CH, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. Pediatrics 2005;115:1-4.

21. Kliegman RM, Willoughby RE. Prevention of necrotizing enterocolitis with probiotics. Pediatrics 2005;115:171-2.

22. Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH. Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet 1994;344: 1046-9.

23. Nopchinda S, Varavithya W, Phuapradit P, Sangchai R, Suthutvoravut U, Chantraruksa V, et al. Effect of *Bifidobacterium Bb12* with or without *Streptococcus thermophilus* supplemented formula on nutritional status. J Med Assoc Thai 2002;85(suppl 4):S1225-31.

24. Cnota J, Shetty AK, Land MH, Rouster-Stevens K, Woods C, Cannon ML. Sepsis associated with probiotic therapy lactobacillus. Pediatrics 2005; 115:178-81.