Original Report

Reduced Incidence of Necrotizing Enterocolitis Associated with Enteral Administration of *Lactobacillus acidophilus* and *Bifidobacterium infantis* to Neonates in an Intensive Care Unit

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ABSTRACT

Objectives: Necrotizing enterocolitis (NEC) has been associated with a wide variety of bacteria and their cytotoxins. The content and the nature of gut bacterial colonization in newborns that require intensive care hospitalization has been demonstrated to be abnormal. In the 25-bed neonatal intensive care unit in Hospital Simon Bolivar, in Bogotá, Colombia, cases of NEC are common causes of morbidity and mortality. This article examines the hypothesis that oral administration of prophylactic *Lactobacillus acidophilus* and *Bifidobacterium infantis* to all neonates in an intensive care unit, would decrease the incidence of NEC.

Methods: Daily doses of 250 million live *L. acidophilus* and 250 million *B. infantis* were given to all 1237 newborns (both inpatients and transfer patients) admitted to the unit during 1 year, until they were discharged from the hospital. In this study, 1282 patients hospitalized during the previous year were used as controls.

Results: There were no complications attributed to the daily administration of *L. acidophilus* and *B. infantis*. The study groups were compared for place of origin, clinical, and demographic variables, and there was no statistically significant difference in those variables. In the historic control group, there were 85 NEC cases compared to 34 cases in the group that received probiotic prophylaxis ($P < 0.0002$). In the historic control group, there were 35 NEC-associated fatalities compared to 14 fatalities in the group that received probiotic prophylaxis ($P < 0.005$).

Conclusions: The positive results in this study support the need for further investigation of bacterial colonization and its role in NEC.

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Although the etiology of necrotizing enterocolitis (NEC) still is not clear, possible contributing factors include pathogenic bacteria, an imbalance of bacteria in the intestinal lumen, and barrier dysfunction. The fact that NEC does not occur in utero in spite of stress and fetal ingestion of 150 mL/kg per day of amniotic fluid that contains proteins, carbohydrates, fat, immunoglobulins, and electrolytes suggests that bacteria are critical in the pathogenesis of this disease.[1,2] Animal experiments also have demonstrated the need for bacterial colonization in NEC.[3–5]

Research examining the content and the nature of gut bacterial colonization in newborns that require intensive care hospitalization has demonstrated that colonization is abnormal. The appearance of heterogeneous microflora is both delayed and replaced by only a few species.[6] A newborn that is colonized with aggressive and drug-resistant bacteria from the neonatal intensive care unit may be at greater risk for NEC or its complications. The use of oral antibiotics modifies gastrointestinal microflora, but the risk for resistant bacteria overgrowth has discouraged its use.[7,8] Studies in adult transplants have demonstrated bacterial translocation with oral antibiotics.[9–11]

The usefulness of biotherapeutic agents (microorganisms with therapeutic properties) in gastrointestinal infections has been reviewed.[12] It has been demonstrated that *Lactobacillus acidophilus*, by adequately adhering to the intestinal wall, can inhibit other pathologic bacteria,[13–15] including *Klebsiella pneumoniae* and *Candida albicans*,[16,17] and the utilization of bifidobacteria to reduce NEC has been demonstrated in rats.[18] However, some trials to modify bacterial colonization by administering *Lactobacillus* and maintaining oral feedings during treatments with antibiotics in neonates have been successful in colonizing the gut with *Lactobacillus*, but without modifying the gram-negative resistant bacterial

colonization.[19] Nevertheless, the production of staphylococci and *Clostridium difficile* endotoxins or the presence of gram positive bacteria in these settings is unknown. This induced colonization has not been evaluated in relation to the incidence of NEC.

Probably secondary to the lack of staff and resources, cases of NEC occurred frequently in the newborn unit of Hospital Simon Bolivar, a public university hospital in the city of Bogotá. These cases were associated with serious outcomes, including fatalities. The NEC cases typically occurred in small clusters; initial interventions with antibiotic treatments and temporary isolation measures in the unit partially controlled them. However, the clusters of NEC soon returned. An alternative approach was developed with the hypothesis that modifying the intestinal microflora colonization of all the newborns in the unit would decrease the incidence of NEC.

METHODS

Setting

Hospital Simon Bolivar is the only third level referral public university hospital for the poor northwest section of the city, and northern part of the state. Nevertheless the hospital receives patients from the entire state. The neonatal intensive care unit is a 25-bed unit grouped into three sections (intensive care, intermediate care, and minimal care) that are not physically divided.

Population and Microorganism Administered

The probiotic agent, Infloran Berna7 (Swiss Serum and Vaccine Institute Berne, Berne, Switzerland) that contained 1000 million live *L. acidophilus* and 1000 million *Bifidobacterium infantis* per capsule was used. All newborns (both inpatients and transfer patients) admitted to the unit during 1 year (October 15, 1994, to October 15, 1995) received prophylactically one-quarter capsule of probiotic agent dissolved in 1 mL of sterile water or 5% dextrose daily during their entire hospital stay. This group is designated probiotic group (PG). If the patient could suction and swallow, the preparation was put directly on the mouth drop by drop, otherwise it was given by orogastric tube. If the newborn had a gastrointestinal problem that required decompressive gastric tube, the stomach was emptied, the preparation was introduced through the tube, the neonate was put in a right lateral position, and the tube was closed for 1 to 3 hours, according to the tolerance for this closure. All neonates were observed carefully for any signs or symptoms attributable to the use of the preparation. Stool color and consistency was evaluated to assess tolerance. Patients hospitalized during the previous year (October 15, 1993 to October 15, 1994) were used as the control group (designated as no-probiotic group: no-PG).

The study was approved by the Research Committee of the Hospital Simon Bolivar. Informed consent was obtained from the parents or guardians.

Definition of Necrotizing Enterocolitis

A case of NEC was defined as a patient with at least one gastrointestinal, one systemic, and one radiographic parameter of the following:

Gastrointestinal criteria:

1. Abdominal distention or tenderness
2. Feeding intolerance that consisted of presence or increase in the gastric aspirates (residues of more than 30% of the administered milk) or persistent emesis
3. Stools with occult or gross blood
4. Diarrhea and erythema of abdominal wall accompanied by systemic signs and symptoms, including (a) lethargy, (b) apnea or respiratory distress, (c) temperature or glucose instability, (d) poor perfusion or shock, (e) disseminated intravascular coagulation, or (f) positive blood cultures that were not present before the gastrointestinal signs and symptoms.

Radiologic criteria:

1. Dilated bowel loops (as compared with the first lumbar vertebra)
2. Bowel wall thickening
3. Intraperitoneal fluid
4. A single persistent dilated loop
5. Pneumatosis intestinalis
6. Portal vein gas
7. Pneumoperitoneum.

The final determination of the diagnosis of NEC was made by a small group of attending physicians that did not change during the study years. If the attending physician at the end of the course considered there was sufficient evidence of NEC, the case was included in the study; if, on the contrary, the physician thought there was not enough evidence it was not included. Both suspected and confirmed NEC were included in both groups.

Feeding Protocol

There is a strict feeding protocol with daily fixed increases that existed in the unit prior to the study and was maintained equally in the 2 years of the study. Depending on the birth weight and gestational age, a fixed amount per kilogram of half strain milk is initiated after one or two trials with still water. It is advanced, if tolerated, with slow careful increases. If there is any sign of intolerance it is immediately stopped. Most premature infants under 1500 g receive total parental nutrition prior to and during advancement until full feedings are tolerated.

The group of physicians in charge of the patients were not modified except by the rotation of residents who follow established protocols in the unit. Neither the nursing nor the other support staff in the unit were changed during the study period or the year before. There were no modifications in any managing protocols, clinical practices, equipment, infrastructure, or any other elements in the unit that could have produced changes in the probiotic group compared with the previous year.

Data Collection

During the 2 years of the study, data collection was done by completing a surveillance record at the time of discharge that included 280 different clinical and demographic variables of general epidemiologic information as well as specific information regarding the patient's condition.

Statistical Analysis

Data were entered into a personal computer by using SAS (version 6.0 SAS Institute Inc. 6.0) (SAS Campus Drive, Cary, North Carolina). Logistic regression analysis was used to assess whether the independent variable studied (probiotic group) correlated with NEC or associated mortality. Patient demographic, clinical, illness, and probiotic group characteristics (e.g., sex, maternal age, parity, birth weight, gestational age, Apgar scores, other infections, different treatments) were covariates. Independent risk factors for NEC were examined in both populations for confounding and interacting variables. The Student's t-test and the Mann-Whitney test for nonparametric variables also were used. A value of $P < 0.05$ was considered statistically significant. Patients born in the institution were compared both together and separately from patients born in other hospitals and transferred.

RESULTS

A total of 1237 newborns received one-quarter capsule of the probiotic agent preparation daily; approximately 9000 doses were administered during the study. The first modification observed was a change in color of the stools from assorted coffee and green tones to light yellow in most of the newborns receiving the preparation.

The study groups were compared for place of origin, demographic and clinical variables (Tables 1 and 2). Owing to the length of the results, the full demographic data are not presented. The only statistically significant difference among these variables was that there were fewer babies in the probiotic group in the 2000- to 2499-g weight category. There also were more babies in the probiotic group under 1500 g; however, this did not reach statistical significance.

Table 1. Comparison of Study Groups by Place of Origin

	Study Group		
	No-Probiotics n = 1282 (%)	*Probiotics n = 1237 (%)*	*P*
Inpatient	935 (72.9%)	918 (74.2%)	NS
Transfer	261 (20.4%)	238 (19.2%)	NS
Readmission	64 (5.0%)	46 (3.7%)	NS
Without data	22 (1.7%)	35 (2.8%)	NS

The incidence of NEC in inpatients treated with probiotic prophylaxis was reduced to one-third of the number of cases in the control group (18 vs. 47 cases; $P < 0.0005$), and the transferred patients who were treated had less than half the number of cases seen in the untreated group (19 vs. 38; $P < 0.03$) (Table 3). The decrease in inpatient NEC-associated mortality was even greater with a decrease from 25 to 7 cases. The incidence of NEC was predominantly in infants with low birth weight in both the probiotic group and the no-probiotic group (65% vs. 69%). The average weight (2040 g vs. 2048 g) and gestational age (35 weeks in both) were similar in the two groups. The inpatients that developed NEC were smaller than the transfer patients (32% vs. 79% above 2000 g) and less mature (25% vs. 48% over 36 weeks gestational age) in the no-probiotic group, but they were similar in weight and maturity in the probiotic group (50% and 53% above 2000 g and 26% vs. 33% over 36 weeks gestational age).

All cases of NEC in 1994 (no-PG) with intestinal perforation secondary to NEC died (6/6), five were inpatients and one was a readmission. In 1995 (PG), two inpatient cases with intestinal perforation secondary to NEC died, the first case secondary to septic shock and coagulopathy and the second case 45 days later secondary to intestinal complications.

All patients with NEC with perforations had surgery during both years. Sometimes it was to place a drain, owing to poor prognosis. All cases with NEC had systemic antibiotics for at least 10 days if they survived. No oral antibiotics were used in either year.

No complications attributed to the use of the probiotic preparation were observed. It is interesting to note the unanimous consensus among the neonatal intensive care unit staff that there was an improvement in feeding tolerance and diaper dermatitis with and without *Candida albicans* infection; however this parameter was not consistently recorded during 1994 (no-PG); therefore, it remains only a general observation. During 2 months in the PG period, there was an increase in systemic candidiasis in patients who received extended antibiotics, including three of the patients with intestinal perforation.

Table 2. Description of Study Population

	Inpatient			*Transfer*		
	No-T n = 935 (%)	*PG n = 918 (%)*	*P*	*No-PG n = 347 (%)*	*PG n = 319 (%)*	*P*
Average hospital stay (d)	5.5	5.6	NS	8.5	8.5	NS
Average birth weight (g)	2594	2600	NS	2630	2746	NS
Less than 1000 g	20 (2)	22 (2)	NS	0 (0)	2 (1)	
1000–1499 g	43 (5)	58 (6)	NS	19 (5)	14 (4)	NS
1500–1999 g	127 (14)	118 (13)	NS	38 (11)	43 (13)	NS
2000–2499 g	245 (26)	198 (21)	< 0.05*	81 (23)	46 (14)	NS
2500–4000 g	460 (49)	485 (53)	NS	188 (54)	187 (59)	NS
More than 4000 g	38 (4)	42 (5)	NS	17 (5)	19 (6)	NS
No data	2 (0)	4 (0)		4 (1)	8 (2)	
Average gestational age (wk)	37	37	NS	37	37	NS
Less than 28 wk	17 (2)	21 (2)	NS	3 (1)	1 (0)	NS
28–32 wk	101 (11)	83 (9)	NS	26 (8)	17 (5)	NS
33–34 wk	90 (10)	78 (8)	NS	20 (6)	19 (6)	NS
35–36 wk	88 (9)	99 (11)	NS	23 (7)	32 (10)	NS
37–40 wk	415 (44)	450 (49)	NS	131 (38)	121 (38)	NS
41–42 wk	138 (15)	119 (13)	NS	22 (6)	22 (7)	NS
More than 42 wk	12 (1)	9 (1)	NS	2 (1)	2 (1)	NS
No data	75 (8)	59 (6)	NS	120 (35)	105 (33)	NS
1 minute Apgar scores						
Average	7	7	NS	7	7	NS
Less than 4 (%)	83 (9)	76 (8)	NS	14 (4)	8 (3)	NS
4–6 (%)	156 (17)	159 (17)	NS	30 (9)	25 (8)	NS
More than 6 (%)	652 (70)	628 (68)	NS	113 (33)	102 (32)	NS
Without data (%)	45 (5)	55 (6)	NS	190 (55)	184 (58)	NS
5 minute Apgar scores						
Average	8	9	NS	9	9	NS
Less than 4 (%)	23 (2)	13 (1)	NS	3 (1)	2 (1)	NS
4–6 (%)	88 (9)	73 (8)	NS	13 (4)	8 (3)	NS
More than 6 (%)	781 (83)	776 (85)	NS	136 (39)	121 (38)	NS
Without data (%)	44 (5)	56 (6)	NS	195 (56)	187 (59)	NS
Twins	61	58	NS	19	22	NS
Sex						
Male (%)	503 (50)	507 (55)	NS	188 (54)	186 (58)	NS
Female (%)	427 (50)	400 (44)	NS	156 (45)	118 (37)	NS
No data (%)	4 (0)	8 (1)		3 (1)	15 (5)	
Ambiguous (%)	2 (0)	3 (0)				
Meningitis	2	8		5	2	
Pneumonia	37	34	NS	89	90	NS
Sepsis	39	36	NS	31	38	NS
Treatments						
Total parenteral nutrition	82	80	NS	58	51	NS
Antibiotics	229	245	NS	166	151	NS
Surgery (gastrointestinal)	11	17	NS	20	16	NS
Plasmapheresis	44	65	NS	16	13	NS
Assistant ventilation	69	73	NS	34	34	NS
General mortality	98	93	NS	42	44	NS

*Statistically significant; NS = statistically nonsignificant.

The evolution of all the cases was satisfactory. *Candida albicans* was cultured from one of the total parenteral nutrition equipment sets.

Whereas there were frequent NEC clusters during 1994, there were only two clusters with gastrointestinal disease during 1995, which began in patients from an adoption house who had diarrhea as the original symptom, and were probably of viral origin. The first cluster only had two cases. The first case did not have NEC, only diarrhea, but a patient in an adjacent bed space did develop NEC. Several months later, another cluster of gastrointestinal disease, involving eight patients, began with a neonate from the same adoption house who had diarrhea. The infant died a few hours later of septic shock and intestinal perforation. This diarrhea epidemic lasted 2 weeks, but there were no further cases of NEC. Although these clusters were probably of viral origin, it was not possible to obtain microbiologic confirmation, because of lack of viral culture and other viral identification techniques. There were no positive bacterial blood cultures in any case during those clusters in 1995.

As an observation, the use of the probiotic preparation was continued in the unit after the study was finished, but secondary to administrative difficulties, 5 months later this protocol was stopped. Soon after, the enterocolitis returned, and there were several fatalities.

Table 3. Incidence of Necrotizing Enterocolitis in Probiotic-Treated Neonates and Controls

	Number of Cases with NEC		
	No-Probiotic	*Probiotic*	*P-Value*
Patient origin	85	37	<0.0002*
Inpatient	47	18	<0.0005*
Transfer	38	19	<0.03*
Patients with NEC number admitted (%)	6.63	2.99	
Necrotizing enterocolitis-associated mortality	35	14	<0.005*
Inpatient	25	7	<0.004*
Transfer	10	7	NS

*Statistically significant; NS = statistically nonsignificant.

CONCLUSIONS

1. There were no complications attributed to the daily oral administration of 250 million *L. acidophilus* and 250 million *B. infantis*.
2. In neonatal intensive care units with limited staff and resources and frequent epidemics of NEC, systematic use of daily oral prophylactic *L. acidophilus* and *B. infantis* may partially or totally control these clusters, reducing cases of NEC and associated mortality.
3. This project raises the casual observation of improved oral feeding tolerance and decreased incidence of diaper dermatitis with and without candidal infection in patients receiving Infloran, that needs further study.

Although positive results were expected, the disappearance of NEC in its cluster form was a surprise. The gastrointestinal clusters in 1995 during the administration of the preparation looked more like a viral infection brought from the adoption house than classic NEC. More mature and larger babies in this study developed NEC compared with the usual tiny babies who developed NEC in the United States. Probably this has to do with the larger average weight of the entire unit population in Bogotá, the percentage of fewer tiny survivors, as well as the inadequate conditions that exposes more mature babies to intestinal colonization with aggressive resistant bacteria in Bogotá.

Many infants required plasmapheresis, because Bogotá is 8500 feet above sea level and polycythemia is common. This was done peripherally without umbilical catheterization. In fact, umbilical catheters were rarely used, and none of the babies that developed NEC were umbilically catheterized. Central lines were common, all of them via jugular vein. This procedure was performed equally in both groups.

Systematic gastrointestinal cultures were not done during the study. Twenty-five infants who received the probiotic agent for at least 5 days were cultured after the study, including 10 of them who received systemic antibiotics. Most of them were colonized with *L. acidophilus* or *B. infantis*, and there was no difference if they were receiving systemic antibiotics, probably because of daily administration of new preparation each morning. Among other microflora 3 of 25 babies also were colonized with *E. coli* and none with *Klebsiella*. There is some incomplete information on colonization of some babies with *Klebsiella*, who developed severe NEC with sepsis both in 1994 and 1995. *Klebsiella*, which usually is resistant to most common antibiotics, is an endemic bacteria in several units in crowded public hospitals in Bogotá, including Hospital Simon Bolivar. Unfortunately incomplete data are available for 1994, precluding any comparison.

The complexity of the pathology in NEC has generated a large number of possible interventions that are difficult to evaluate with strict scientific rigor. This is not a double-blind placebo-controlled study. This methodology was designed to obtain groups that are comparable, with the only difference being the intervention. Fortunately, the two groups in this study are comparable, as the statistical analysis of a large number of variables indicates. The positive results in this study highlight the need for further investigation into bacterial colonization and its role in NEC. Several questions emerge as a result of this study. If the alteration of gastrointestinal flora by the administration of supplements is useful in neonatal intensive care units with limited resources, is it also useful for units with much better control of nosocomial infections? Furthermore, although prior studies indicate that adequate colonization by nonpathogenic bacteria might improve the tolerance to oral feeds and avoid "luminal starvation," this must be documented in adequately controlled studies.

Speculation

Despite the theoretic benefits of inducing colonization with nonpathogenic bacteria in neonates, there has been resistance to administering microorganisms to neonates. Consequently, it has been difficult to study these types of interventions in newborn intensive care units with sufficient patients and a variety of microorganisms. How does altering gut colonization decrease NEC? The ecologic principle of an adequate balance of the live organisms may be applicable. The replacement of resistant and

aggressive bacteria with nonpathogenic strains in total number of organisms as well as possibly reducing the toxin-producing strains may be one of the principle mechanisms. Alternatively, there may be a more complex interaction with a combination of several types of microflora that modify the adherence or invasiveness of the possible causative organisms of NEC.[20] However, it also is plausible that the type of gut colonization of the newborn is a determinant of the intensity of the inflammatory processes that can be produced by the interaction of bacteria with the oral feedings.

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