Effect of Oral Probiotics Supplementation in the Prevention of Necrotizing Enterocolitis among Very Low Birth Weight Preterm Infants

Varaporn Saengtawesin MD*,
Raungpung Tangpolkaiwalsak MD*, Wiboon Kanjanapattankul MD*

** Neonatal Unit, Department of Pediatrics, Queen Sirikit National Institute of Child Health, College of Medicine, Rangsit University, Bangkok, Thailand*

Background: *Necrotizing enterocolitis (NEC) is the most serious gastrointestinal problem in very low birth weight preterm infants. Multiple risk factors activate the inflammatory cascade leading to high expressions of pro-inflammatory mediators causing bowel injury in NEC. The anti-inflammatory effect of probiotics is due to the inhibition and reduction of inflammatory signal in intestinal epithelium.*

Objective: *To evaluate the efficacy of probiotics supplementation in the prevention of NEC among very low birth weight preterm infants.*

Study design: *A prospective randomized controlled trial.*

Material and Method: *All preterm infants with gestational age less than or equal to 34 weeks and birth weight less than or equal to 1,500 grams admitted in neonatal care unit, Queen Sirikit National Institute of Child Health during June 1st, 2012 and January 31th, 2013 were enrolled in this study. They were randomized into two groups, study and control group. Infants in the study group were fed Infloran® (Lactobacillus acidophilus $1x10^9$ and Bifidobacterium bifidum $1x10^9$ organisms) dose 125 mg/kg/dose twice a day with breast milk or premature formula from the start of feeding until 6 weeks or discharge. Infants in the control group were fed with either breast milk or premature formula alone. The primary outcome was NEC stage ≥ 2.*

Results: *Sixty infants completed the study, 31 infants in the study group and 29 infants in the control group. The baseline characteristic data of infants were similar, except for more males in the present study group. Incidence of NEC stage ≥ 2 were similar in both the groups, 3.2 vs. 3.4% (p = 0.74). There were no deaths during the study period. Days to reach full feeding, 150 ml/kg/day, were no differences between the two groups, 12.03 ± 5.49 days vs. 13.76 ± 8.25 days (p = 0.31). No adverse effects such as sepsis, flatulence or diarrhea were noted.*

Conclusion: *In this study, there was no difference in incidence of NEC stage ≥ 2 between the two groups. No adverse effects of probiotics supplementation were observed.*

Keywords: *Oral probiotics, Prevention, Necrotizing enterocolitis, Very low birth weight*

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Necrotizing enterocolitis (NEC) is the most serious gastrointestinal problem in very low birth weight preterm infants, affecting 7-14% of preterm infants[1,2]. Common factors associated with NEC are prematurity, enteral feeding, intestinal ischemia and bacterial colonization[1,3-5]. However, NEC has multiple risk factors which results in the activation of inflammatory cascade leading to high expressions of pro-inflammatory mediators resulting in bowel injury[3-5]. Probiotics are live non-pathogenic microbials present in the human gastrointestinal tract and provide benefit to the host[6-8]. Probiotic microorganisms generally consist of various strains of Lactobacillus, Bifidobacterium and Streptococcus[9]. Probiotics normalize intestinal microflora, increase mucosal barrier function, reduce intestinal permeability, enhance immune defenses and improve enteral nutrition leading to reduction in bacterial translocation[10]. Probiotics exhibit their anti-inflammatory effects by inhibiting or reducing inflammatory signal in intestinal epithelium.

Because probiotics can modify the occurrence of inflammatory cascade, probiotics may reduce incidence of NEC in very low birth weight preterm infants.

Correspondence to:
Sangtawesin V, Neonatal Unit, Departmet of Pediatrics, Queen Sirikit National Institute of Child Health, College of Medicine, Rangsit University, Bangkok, Thailand.
Phone: 0-2354-8439
E-mail: varanewborn@gmail.com

Material and Method

A prospective, randomized controlled trial was conducted in the neonatal care unit of Queen Sirikit National Institute of Child Health. From June 1, 2012 to January 31, 2013 all preterm infants with gestational age less than or equal to 34 weeks and birth weight less than or equal to 1,500 grams were randomized by blocks of four into two groups, study and control group. Very low birth weight preterm infants who had severe birth asphyxia, chromosome anomalies, cyanotic congenital heart disease, congenital intestinal obstruction, gastroschisis, omphalocele, nil per oral >3 weeks and parents who declined consent for study were excluded from the study. Infants who were randomized in the study group were fed Infloran®. One capsule of Infloran® consisted of $1x10^9$ Lactobacillus acidophilus and $1x10^9$ Bifidobacterium bifida. The dose of Infloran® was 125 mg/kg/dose twice a day. Infloran® was fed with either breast milk or premature formula from the day feeds were started until 6 weeks or discharge. Infants in the control group were fed either breast milk or premature formula alone. Infloran® was stored in a refrigerator at 4°C to 8°C, at the hospital pharmacy and sent then to the neonatal unit according to prescription. One capsule of Infloran® was dissolved with 1 ml sterile water in personalized sterile glass and the calculated dose of Infloran® was given via orogastric tube with feeds to infants. Daily supplements were continued until 6 weeks or discharge.

Whenever an infant was suspected to have NEC, he/she was evaluated by two attending neonatologists. Clinical status and abdominal film were reviewed and NEC was categorized by modified Bell's classification[11]. Spontaneous intestinal perforation (SIP) also was evaluated by two neonatologists and diagnosed by clinical status, abdominal film and pathology.

Statistical analysis

Mean ± SD and Student's t-test were used to analyze continuous data. The x^2 test was used to analyze categorical data, along with Fisher's exact test.

The primary outcome of interest was efficacy of probiotics supplementation in the prevention of NEC stage ≥2 among very low birth weight preterm infants. Secondary outcomes were time to complete enteral feeding (100 ml/kg/day) and adverse effects from probiotics supplement, such as Lactobacilllus or Bifidobacterium sepsis, flatulence or diarrhea[1].

Results

There were 97 VLBW infants admitted in the neonatal care unit of Queen Sirikit National Institute of Child Health during June 1st, 2012 and January 31th, 2013. Of these 97 infants, 8 cases had severe birth asphyxia, 5 cases had major congenital anomalies, 1 case had gastroschisis, 17 cases had incomplete data and parents declined consent for study in 6 cases. Sixty infants were enrolled in the study, 31 infants were in the present study group and 29 infants were in control group. There were no differences in the maternal and infant clinical characteristics between the two groups (Table 1). Majority of infants in study group were male, 19 of 31 (61.3%) when compared to control group, 11 of 29 (37.9%) (Table 1). The infant's clinical variables did not differ between the two groups, except for more use of antibiotic at 2 weeks age in control group (Table 2). Table 3 shows the primary outcomes of the study. The incidence of NEC stage ≥2 did not differ between the two groups (1 of 31 infants vs. 1 of 29 infants). There

Table 1. Maternal clinical and infant's demographic and clinical characteristics

Variable	Study (n = 31)	Control (n = 29)	*p*-value
GA, mean ± SD (week)	31.0±1.82	30.59±1.76	0.34
Birth weight, mean ± SD (gram)	1,250.1±179.26	1,207.72±199.35	0.49
PROM, n (%)	10 (32.2)	7 (24.1)	0.49
Chorioamnionitis, n (%)	0	0	1.00
Preeclampsia, n (%)	1 (3.2)	4 (13.8)	0.14
Prenatal steroid treatment, n (%)	23 (74.2)	24 (82.7)	0.42
Prenatal antibiotics, n (%)	18 (58)	17 (58.6)	0.96
Cesarean section, n (%)	21 (67.7)	18 (62)	0.64
Male, n (%)	19 (61.3)	11 (37.9)	0.03*
SGA, n (%)	3 (9.7)	2 (6.9)	0.69
Apgar scores of <6 at 5 min, n (%)	1 (3.2)	1 (3.2)	0.96

were no deaths in the entire cohort neonates during the study period. The secondary outcomes are shown in Table 4. There was no difference in time for full enteral feeding between the two groups. There were also no differences in the feeding amount and weight gain in both groups (Table 4). There were no differences in other outcomes, such as sepsis, bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH) stage ≥3, periventricular leukomalasia (PVL) and retinopathy of prematurity (ROP) stage ≥3 in both groups (Table 5).

Discussion

From the present study of effect of oral probiotics supplementation in VLBW infants there was no difference in the incidence of NEC stage ≥2 between the study and control groups. There were no infant deaths of those diagnosed with NEC stage ≥2 in either group.

The result of the present study may differ from many previous studies that have showed probiotics supplementation to reduce the incidence of NEC among VLBW preterm infants[1,12-14]. This difference may be due to the number of infants enrolled, which was much lower than a calculated number needed to show a significant difference.

The development of NEC is multifactorial and has been associated with factors such as preterm labor, preterm delivery and infection but so far, there has been no association between gender and incidence of

Table 2. Clinical variables in study infants

Variable	Study (n = 31)	Control (n = 29)	*p*-value
Use of surfactant, n (%)	4 (12.9)	4 (13.8)	0.92
PDA with ibuprofen treatment, n (%)	12 (38.7)	15 (51.7)	0.31
Antibiotics at 2 week, n (%)	27 (87)	18 (62)	0.03
Antibiotics at 4 week, n (%)	14 (45.1)	14 (48.3)	0.81
Antibiotics at 6 week, n (%)	4 (12.9)	8 (27.6)	0.16
Ventilator uses, median (interquatile)	6 (3-14)	6 (3-13)	0.75
Length of stay, mean ± SD (day)	59.84±31.96	56.9±27.19	0.80
EBM, n (%)	12 (38.7)	11 (37.9)	0.95
Mixed feeding, n (%)	19 (61.3)	18 (62.1)	0.73
UAC use, n (%)	25 (80.6)	21 (72.4)	0.45
UVC use, n (%)	31 (100)	28 (96.6)	0.29

EBF = exclusive breast milk; Mixed feeding = breast milk and premature formula milk; UAC = umbilical arterial catheter; UVC = umbilical venous catheter

Table 3. Outcome variables of probiotics supplementation

Variable	Study (n = 31)	Control (n = 29)	*p*-value
NEC stagee ≥2, n (%)	1 (3.2)	1 (3.4)	0.74
Death attributable to NEC, n (%)	0	0	-
Death not attributable to NEC, n (%)	0	0	-

Table 4. Feeding amount and weight gain with probiotics supplementation

Variable	Study (n = 31) Mean ± SD	Control (n = 29) Mean ± SD	*p*-value
Feeding amount at 42 days (ml/kg/day)	144.77±15.36	146.59±16.78	0.32
Full feeding day (day)	12.03±5.49	13.76±8.25	0.64
Weight gain at 14 days (gram)	76.55±63.93	81.38±95.71	0.68
Weight gain at 28 days (gram)	359.1±173.24	360±172.9	0.88
Weight gain at 42 days (gram)	632.45±281.19	551±333.1	0.21

Table 5. Other variable outcomes

Variable	Study (n = 31)	Control (n = 29)	*p*-value
Early onset sepsis, n (%)	0	0	-
Late onset sepsis, n (%)	2 (6.45)	1 (3.44)	0.53
BPD, n (%)	16 (51.6)	10 (34.4)	0.18
IVH grade ≥3, n (%)	3 (9.6)	0	0.09
PVL, n (%)	0	1 (3.4)	0.29
ROP stage ≥3, n (%)	1 (3.2)	0	0.33

NEC[15]. Although there were more males in the present study than in the control group, there were no differences in the incidence of NEC between either of the groups.

Breast milk has many components that are thought to be protective against NEC such as immunoglobulin, lysozyme, lactoferin and PAF-acetyl transferase[16]. Infants in both the groups had equal number of exclusive breastfeeding rates of 38% and interestingly both the cases of NEC stage ≥2 diagnosed in the present study were from the mixed feeding group. This information supports the benefit of breast milk in preventing NEC.

A major fear in the use of probiotics in the VLBW was that it might increase sepsis. In a study published by Lin et al, probiotics were shown to reduce the incidence of sepsis in VLBW infants[1]. The current study, however, did not show any difference in incidence of sepsis between the two groups. This may be that sepsis is a complex disorder involving many factors. Probiotics alone cannot overcome all factors that cause sepsis. Moreover, none of the three infants with late onset sepsis had Lactobacillus or Bifidobacterium bacteremia. Other morbidities like BPD, IVH, PVL and ROP were also not affected by probiotics supplementation. This may explained by the primary effect of probiotics on the gastrointestinal system.

Conclusion

There was no difference in incidence of NEC stage ≥2 from oral probiotics supplementation in VLBW pre-term infants in the present study. No adverse effects of probiotics supplementation such as sepsis, flatulence or diarrhea were observed. However, larger studies need to be conducted in the future.

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Potential conflicts of interest

None.

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ผลของการเสริมโปรไบโอติกเพื่อป้องกันการเกิดภาวะลำไส้อักเสบติดเชื้อในทารกเกิดก่อนกำหนดน้ำหนักตัวน้อยมาก

วราภรณ์ แสงทวีสิน, รวงผึ้ง ตั้งผลไกวัลศักดิ์, วิบูลย์ กาญจนพัฒนกุล

ภูมิหลัง: ภาวะลำไส้อักเสบติดเชื้อเป็นปัญหารุนแรง ซึ่งมีอัตราตายและความพิการสูงในทารกเกิดก่อนกำหนดน้ำหนักตัวน้อยมาก ปัจจัยเสี่ยงการเกิดมีหลายสาเหตุทำให้เกิด pro-inflammatory mediators ในลำไส้เป็นผลทำให้เกิดการอักเสบติดเชื้อของเนื้อเยื่อภายในลำไส้

วัตถุประสงค์: ศึกษาผลของการเสริมโปรไบโอติก กินเพื่อป้องกันการเกิดภาวะลำไส้อักเสบติดเชื้อในทารกเกิดก่อนกำหนดน้ำหนักตัวน้อยมาก

วัสดุและวิธีการ: A prospective randomized controlled trial ทารกเกิดก่อนกำหนดอายุครรภ์น้อยกว่าหรือเท่ากับ 34 สัปดาห์ และน้ำหนักน้อยกว่าหรือเท่ากับ 1,500 กรัม ที่เข้ารับการรักษาในสถาบันสุขภาพเด็กแห่งชาติมหาราชินี ระหว่างเดือนมิถุนายน พ.ศ. 2555 ถึง เดือนมกราคม พ.ศ. 2556 แบ่งออกเป็น 2 กลุ่มโดยการสุ่ม กลุ่มทดลองได้โปรไบโอติกชนิด Lactobacillus acidophilus 1,000 ล้านตัว และ Bifidobacterium bifidum 1,000 ล้านตัว ขนาด 125 มิลลิกรัม ต่อน้ำหนักกิโลกรัมต่อครั้ง วันละสองครั้ง ผสมในนมแม่หรือนมแม่ผสมกับนมผง เริ่มกินตั้งแต่กินนมมื้อแรก กินนาน 6 สัปดาห์ หรือจนกว่าจำหน่ายออกจากโรงพยาบาล กลุ่มควบคุมคือไม่ได้โปรไบโอติกเสริม โดยติดตามผลลัพธ์จากอุบัติการณ์การเกิด NEC stage ≥2

ผลการศึกษา: ทารกเข้าร่วมการศึกษาทั้งหมด 60 ราย เป็นกลุ่มทดลอง 31 รายและกลุ่มควบคุม 29 ราย ลักษณะข้อมูลพื้นฐานของทารกทั้งสองกลุ่มยกเว้นเพศไม่มีความแตกต่างกัน อุบัติการณ์การเกิด NEC stage ≥2 ระหว่างกลุ่มทดลองและกลุ่มควบคุมเท่ากับร้อยละ 3.2 และร้อยละ 3.4 ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ (p = 0.74) ไม่พบการตายจากงานวิจัยนี้ ไม่พบความแตกต่างในวันที่สามารถกินนมได้เต็มที่ 100 มิลลิลิตร ต่อกิโลกรัมต่อวัน ระหว่างสองกลุ่มเท่ากับ 12.03±5.49 วัน และ 13.76±8.25 วัน ตามลำดับ (p = 0.31) ไม่พบผลข้างเคียงจากโปรไบโอติก เช่น ท้องอืด ถ่ายเหลว และภาวะติดเชื้อในกระแสเลือด

สรุป: การศึกษานี้ไม่พบความแตกต่างของอุบัติการณ์การเกิด NEC stage ≥2 และอาการไม่พึงประสงค์ในกลุ่มที่ให้ยาโปรไบโอติกและกลุ่มควบคุม