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ORIGINAL ARTICLE

Effect of early administration of probiotics on gut microflora and feeding in pre-term infants: a randomized controlled trial

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Abstract

Background: This study aimed to evaluate the effect of early probiotic administration on gut microflora and influence on feeding in pre-term infants.
Methods: A double-blind, randomized, controlled clinical study was conducted to assess the effect of probiotics [live, combined lactobacillus and bifidobacterium (LCB)] supplementation in pre-term infants. Sixty hospitalized pre-term babies were randomly assigned to two groups: a probiotics-supplemented group and the control group. The primary endpoint was measurement of lactobacillus and bifidobacterium in the gut. The secondary outcome was the rate of feeding intolerance.
Results: In the first weekend, the quantity of gut lactobacillus and bifidobacterium was significantly higher in the probiotics-supplemented group than in the control group [7.84 ± 0.35 versus 6.39 ± 0.53 (log copy number/g wet fecal weight), $p = 0.013$; 8.52 ± 0.23 versus 7.01 ± 0.48, $p = 0.024$, respectively]. In the second weekend, the amount of gut lactobacillus and bifidobacterium in the probiotics-supplemented group remained significantly higher (8.62 ± 0.28 versus 7.34 ± 0.59, $p = 0.036$ and 9.45 ± 0.64 versus 7.85 ± 0.43, $p = 0.007$, respectively). Fewer patients in the probiotics-supplemented group developed a feeding intolerance (13.3% versus 46.7%, $p = 0.013$).
Conclusions: Probiotic supplementation in the hospitalized pre-term infants in the first 2 weeks of life resulted in higher amounts of lactobacillus and bifidobacterium in the gut and a concomitant lower rate of feeding intolerance.

Keywords

Feeding intolerance, gut microbiota, pre-term infants, probiotic supplementation

History

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Introduction

Microbiological programming of the human gut begins *in utero* and proceeds gradually during birth and infancy [1]. In hospitalized pre-term infants, multiple factors influence the intestinal flora, including the establishment of beneficial bacterial flora and presence of pathogens [2]. The studies focused on normalizing intestinal flora, improving feeding intolerance, prevention of necrotizing enterocolitis and sepsis [3–7]. Despite some encouraging results from probiotic supplementation, the optimal probiotic treatment for this vulnerable population remains unknown. It is critical to investigate the optimal strain(s), dose and time of administration for such probiotics, so that they can be routinely given to pre-term infants [8–10]. In this context, we aimed to investigate whether the early administration of oral probiotics, containing *Lactobacillus acidophilus* and *Enterococcus faecalis* was beneficial to pre-term infants.

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Methods

Patients

The study included 60 pre-term infants admitted to the neonatal intensive care unit (NICU) of the First People's Hospital in Kunshan, Jiangsu University from June to December 2013. The inclusion criterion was: gestational age <35 weeks. Those with serious infections, and necrotizing enterocolitis (NEC) prior to therapy and congenital intestinal malformations were excluded. Ethical approval was obtained from the Research Ethics Committee of the First People's Hospital in Kunshan, Jiangsu University and the study was registered at the following website: http://www.clinicaltrial.gov (ClinicalTrials.gov Identifier: NCT02060084). Informed consent was obtained from all parents. Patient anonymity was preserved.

Randomization

The study was a double-blind, randomized, controlled clinical trial. The patients were randomly assigned to either a control group or a probiotic-supplemented group in the following manner. Treatment-assignment cards were created with a unique randomization code and placed in sequentially

numbered, opaque envelopes. At each site, the cards were pulled in sequential order and the randomization number was used to assign the patient to a treatment group. All investigators, parents, physicians and nurses involved in patient care were blinded to the assignment. The randomization schedule was made available only to the pharmacist who supervised the quality, transport and storage of LCB. Infants were followed until they were discharged from the hospital or died. They were withdrawn from the trial if severe adverse effects developed, or parents withdrew consent. The probiotic-supplemented group was orally administered LCB (Bifico, Shanghai Xinyi Pharmaceutical Inc., Shanghai), and the control group was fed with the same dose of lukewarm water); both the preparations were supplied in identical containers. LCB was orally administered starting from the second day after birth, at a dose of 0.5 g (the numbers of live Long Bifidobacterium, *Lactobacillus acidophilus* and *Enterococcus faecalis* was $>0.5 \times 10^7$ CFU), twice per day, for 2 weeks. In our NICU, the breast milk bank had not been set up and most of the infants were transferred from other hospitals. As parents would not send expressed milk every day, all pre-term infants were fed standard pre-term formula (SPF, NeoSure, Similac) from the first day of life until discharge and feeding was stopped if they developed symptoms of NEC.

Fecal sample collection

Fresh patient fecal samples were collected on days 3, 7 and 14. The samples were stored at $-80\,^{\circ}C$ until further measurement of fecal bacterial DNA.

Observations and measurements

We recorded the start of feeding time, full enteral feeding time, time taken to regain birth weight, days of excreted meconium and weight gain. The incidence, duration and remission of feeding intolerance were recorded.

Monitoring of feeding intolerance and other clinical parameters

During the study, we monitored feeding intolerance (higher osmotic load causing abdominal distension, diarrhea or vomiting), probiotic sepsis and adverse effects (flatulence, loose stools) of additives such as prebiotic oligosaccharides [9]. Feeding intolerance was diagnosed if any of the following symptoms occurred: (1) frequent vomiting (>3 times/day); (2) unchanged or reduced feeding volume (lasting longer than 3 days); (3) gastric retention (>30% of the previous feeding volume); (4) abdominal distention; (5) ''coffee ground'' vomiting; (6) feeding volume <8 ml/kg each time at the end of week 2 and (7) fasting for >2 days.

Real-time quantitative polymerase chain reaction (PCR) for fecal bacterial detection

Fecal bacterial DNA preparation

Bacterial genomic DNA from samples was prepared using the QIAamp DNA stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Design and synthesis of PCR primers and probes

Specific primers and probes targeting lactobacillus and bifidobacteria were designed according to the corresponding 16SrDNA and 16S-23rRNA sequences, and were selected after a Genbank search using BLAST (www.ncbi,nlm,nih.gov/BLAST). Primers and probes were synthesized by Shanghai Shanjing Biotechnology Corporation (Shanghai).

Florescent quantitative PCR

SYBR green I was used to determine the amount of lactobacillus and bifidobacteria. Amplification conditions were as follows: the reaction program for lactobacillus was 95 °C for 15 s, 60 °C for 1 min and 72 °C for 45 s for 40 cycles. The reaction program for bifidobacterium was 95 °C predenaturation for 5 min, followed by 60 °C for 1 min and 72 °C for 45 s for 40 cycles. Real-time quantitative PCR was performed in triplicate using the LightCycle480 (Roche, Basel, Switzerland). Negative controls were included in each assay. Bacterial DNA was accurately quantified and prepared in a 10-fold serial dilution to generate a standard curve.

Statistical analyses

Data were analyzed using SPSS 21.0 (Chicago, IL) for Windows software and are expressed as mean ± SD or median and interquartile range. Between-group differences were analyzed using the *t* test. Differences in rates were analyzed by the chi-squared test. $p<0.05$ was considered to be statistically significant.

Results

General characteristics of the study subjects

A total of 60 pre-term infants met the inclusion criteria, with 30 in the experimental group and 30 in the control group. The gestational age ranged between 30 and 35 weeks with a mean age of 32.3 ± 2.2 weeks. Birth weight of patients ranged between 1530 and 2750 g with a mean birth weight of 1623 ± 422 g. There were no significant differences in sex, birth weight and gestational age between the groups ($p>0.05$) (Table 1). Three patients were withdrawn from the study, two from the experimental group and one from the control group because of severe symptoms of feeding intolerance.

Table 1. Clinical characteristics of two groups.

Group	Experimental group	Control group	*p* values
N	30	30	
M:F	17:13	16:14	
Gestation (weeks)	32.4 ± 1.6	32.1 ± 1.9	0.932*
Birth weight (g)	1653 ± 476	1532 ± 412	0.756*
Cases of ventilation (*n*)	10	7	0.390†
Days of PN (days)	6.7 ± 0.7	8.7 ± 1.0	0.223*
Cases of antibiotics use (*n*)	25	26	0.718†

PN, parenteral nutrition.

**t* Test.

†Chi-squared test.

Feeding intolerance

The rate of feeding intolerance in the experimental group was significantly lower than in the control group (13.3% versus 46.7%, $p = 0.013$). There were no significant differences in daily weight gain between the groups. The total number of days of meconium excreted was significantly shorter in the experimental group than in the control group ($p = 0.032$, Table 2).

Comparison of gut microbiota between the two groups

There were no significant differences in the quantities of gut lactobacillus and bifidobacterium detected between the two groups at day 3 (Table 3). In the first weekend, the amounts of gut lactobacillus and bifidobacterium were higher in the experimental group than in the control group (7.84 ± 0.35 versus 6.39 ± 0.53 $p = 0.013$; 8.52 ± 0.23 versus 7.01 ± 0.48, $p = 0.024$, respectively). In the second weekend, the quantities of gut lactobacillus and bifidobacterium remained significantly higher in the experimental group (8.62 ± 0.28 versus 7.34 ± 0.59, $p = 0.036$; and 9.45 ± 0.64 versus 7.85 ± 0.43, $p = 0.007$, respectively).

Discussion

The present study has demonstrated that the quantities of gut lactobacillus and bifidobacteria were significantly higher in probiotic-supplemented groups at the first and second weekend of pre-term baby life. The significantly lower rate of feeding intolerance in the experimental group further corroborates the positive role of probiotics in reducing feeding intolerance in a selected pre-term population.

Establishment of newborn gut microbiota is complicated and results from a combination of effects of nutritional, immune and environmental factors [2]. Factors such as gestation age, delivery route, feeding methods, living environment, hospital stay and history of antibiotic use determine the content and composition of gut microbiota. Establishment of gut microbiota in pre-term newborns is different from that in full-term newborns [3]. In pre-term newborns, the establishment of gut microbiota is late and less diverse than in full-term newborns; specifically there is a lack of lactobacillus and bifidobacterium. The benefits of healthy bacteria in the human body are numerous and include increased barrier function of the gut wall, better balance of the microbiological environment, prevention of pathogen invasion of the gut wall by commensals attaching to the gut mucosal surface, promotion of protein and enzyme metabolism of food and improvement of epithelial function of the gut mucosa to lower gut permeability [10–12]. *Lactobacillus rhamnosus* and *Bifidobacterium lactis* are commonly used probiotic strains in pre-term newborns [13]. The present study examined fecal microbiota and quantified bacterial contents using florescent quantitative real-time PCR in pre-term newborns who were given oral LCB [14]. The significantly higher contents of gut lactobacillus and bifidobacterium in pre-term newborns who were taking oral LCB at weeks 1 and 2 than those in the control group is consistent with the results from a previous study [15]. There was no significant difference in the contents of gut lactobacillus and bifidobacterium between the two groups on day 3. This might be because of insufficient time for LCB to affect establishment of gut microbiota in such a short time.

Some studies have reported that supplementary probiotics are important for establishment of gut microbiota in newborns [3,4,7]. This establishment of gut microbiota in pre-term newborns appears to be delayed, specifically in those who are hospitalized and have a history of antibiotic use compared with full-term newborns. Among all the bacteria in normal infant gut microbial flora, bifidobacterium and lactobacillus are dominant, and the quantity and quality of these bacteria play an important role in infant gut physiology and health. In the present study, the end date of passing meconium was significantly earlier and the rate of feeding intolerance was significantly lower in the LCB treatment group compared to the control group. These findings are consistent with the earlier report, which showed that oral administration of micro-ecological agents significantly reduced feeding intolerance among very low birth weight newborns [16,17]. Reduced

Table 2. Comparison of meconium excretion, weight gain and rate of feeding intolerance between the two groups.

	Experimental group ($n = 30$)	Control group ($n = 30$)	Statistics	p values
Days of meconium exhausted (days)	4.1 ± 0.6	5.8 ± 1.1	2.345*	0.032
Weight gain (g/day)	22.5 ± 3.6	21.6 ± 4.3	1.358*	0.857
Rate of feeding intolerance (case %)	5 (33.3)	14 (46.7)	6.239†	0.013

*t test.

†Chi-squared test.

Table 3. Comparison of the amounts of fecal lactobacillus and bifidobacterium (log copy number/g wet fecal weight) between two groups at different age.

	Lactobacillus			Bifidobacterium		
Group	Day 3	Week 1	Week 2	Day 3	Week 1	Week 2
Experimental group ($n = 30$)	5.31 ± 0.47	7.84 ± 0.35	8.62 ± 0.28	6.43 ± 0.56	8.52 ± 0.23	9.45 ± 0.64
Control group ($n = 30$)	5.40 ± 0.32	6.39 ± 0.53	7.34 ± 0.59	6.03 ± 0.26	7.01 ± 0.48	7.85 ± 0.43
t value	0.397	3.422	2.016	0.384	2.253	6.783
p value	0.463	0.013	0.036	0.335	0.024	0.007

feeding intolerance might be due to the establishment of healthy gut bacteria, which possess multiple enzymes, boosts digestion and produces a large amount of organic acids during metabolism to stimulate intestinal wall movement and gastric emptying [18].

The limitations of the study include a small sample size and the study being conducted in a single center. We also did not conduct a multivariate analysis for the reduction of feeding intolerance due to the small sample size.

In conclusion, the present study reinforces the role of healthy gut microbial flora in pre-term newborns. Among hospitalized pre-term newborns the supplementation with bifidobacterium and lactobacillus increased the concentration of these organisms in the gut compared to those who were not given LCB. Early supplementation of LCB probably helped to establish healthy bacteria early and stimulated gastric emptying/meconium excretion resulting in reduced feeding intolerance. Therefore, this treatment might be beneficial for hospitalized pre-term newborns. However, the long-term effect of this treatment requires further investigation in larger cohorts.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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