Clinical Nutrition 35 (2016) 802–811

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized control trials

Probiotics and growth in preterm infants: A randomized controlled trial, PREMAPRO study☆

Stephane Hays [a, b], Aurélien Jacquot [c], Hélène Gauthier [d], Christian Kempf [e], Anne Beissel [d], Odile Pidoux [c], Estelle Jumas-Bilak [f], Evelyne Decullier [g], Emmanuelle Lachambre [h], Laurence Beck [h], Gilles Cambonie [c], Guy Putet [a, i], Olivier Claris [d, i], Jean-Charles Picaud [a, b, i, *]

[a] Department of Neonatology, Hôpital de la Croix Rousse, Hospices Civils de Lyon, F-69004 Lyon, France
[b] Rhone-Alpes Human Nutrition Research Center, F-69310 Pierre-Bénite, France
[c] Department of Neonatology, Hôpital A. de Villeneuve, F-34090 Montpellier, France
[d] Department of Neonatology, Hôpital Femme-Mère-Enfant, Hospices Civils de Lyon, F-69500 Bron, France
[e] CK Consulting, Ottrott, France
[f] Department of Bacteriology, UMR5119, Université Montpellier 1, F-34090 Montpellier, France
[g] Department of Methodology and Statistical Analysis, Hospices Civils de Lyon, F-69003 Lyon, France
[h] Nestlé France, Marne-la-Vallée, France
[i] Université Claude Bernard Lyon 1, F-69100 Villeurbanne, France

ARTICLE INFO

Article history:
Received 26 October 2014
Accepted 18 June 2015

Keywords:
Prematurity
Gut microflora
Safety
Sepsis
Necrotizing enterocolitis

SUMMARY

Background & aims: Recent studies have suggested that the gut microflora has metabolic effects. We aimed to evaluate postnatal growth in preterm infants who received different probiotic supplements, and to assess the safety of probiotic administration.

Methods: This prospective, randomized, double-blind, controlled trial was performed at three tertiary care neonatal units. Preterm infants were randomly assigned to receive daily supplementation over 4–6 weeks with placebo (group C) or probiotics (group P). Group P comprised three subgroups: P1 received *Bifidobacterium lactis*, P2 received *Bifidobacterium longum*, and P3 received *B. lactis* and *B. longum*. We assessed postnatal growth during the supplementation period and up to a corrected gestational age (GA) of 41 weeks when body composition was assessed using whole-body dual-energy X-ray absorptiometry. Aerobic and anaerobic blood cultures were performed on suspicion of late-onset sepsis.

Results: The study comprised 199 preterm infants with a mean GA of 29.1 ± 1.4 weeks and a mean birth weight of 1173 ± 210 g, who received a placebo (group C, n = 52) or probiotics (group P, n = 147) from the first week of life. At the end of the supplementation period, no statistically significant differences were seen between the groups in relation to the mean body weight (group C = 1906 ± 23 g, group P = 1875 ± 14 g, p = 0.25), length, or head circumference. The incidence rates of necrotizing enterocolitis and late-onset sepsis were similar in the two groups. At the corrected GA of 41 weeks, there were no differences between the groups with respect to anthropometric measurements or body composition analysis.

Conclusions: Preterm infants receiving *Bifidobacterium* supplements did not exhibit better postnatal growth compared with those who received placebo treatment. No adverse effects were associated with probiotic administration.

Registered under ClinicalTrials.gov Identifier no. NCT01379417.

Abbreviations: ANCOVA, analysis of covariance; DES, day at the end of supplementation; DEXA, dual energy X-ray absorptiometry; GA, gestational age; HC, head circumference; HM, human milk; ITT, intention to treat; NEC, necrotizing enterocolitis; PCR, polymerase chain reaction; PP, per protocol; SD, standard deviation; TT, theoretical term; VLBW, very low birth weight.

☆ This study was supported by Nestle France (Marne-la-Vallée, France) and Nestec (Vevey, Switzerland).

* Corresponding author. Neonatologie, Hopital de la Croix Rousse, 103 Grande Rue de la Croix Rousse, 69004 Lyon, France. Tel.: +33 472 001 550; fax: +33 472 004 125.
E-mail address: jean-charles.picaud@chu-lyon.fr (J.-C. Picaud).

http://dx.doi.org/10.1016/j.clnu.2015.06.006

1. Introduction

Optimal postnatal growth is essential for very low birth weight (VLBW) infants. Indeed, extra-uterine growth restriction is related to complications associated with prematurity and to deficits in nutrient intakes. Recent studies have suggested that aggressive nutritional support can help to reduce weight and length deficits upon discharge from hospital [1].

Probiotics, including *Bifidobacterium*, significantly reduce the incidence of necrotizing enterocolitis (NEC) and mortality rates in VLBW infants [2]. Furthermore, a positive effect on weight gain has been reported in rapidly growing animals [3], which could be associated with the metabolic effects of probiotics [4]. Some authors have even suggested that there might be a relationship between the composition of the gut microflora and a later risk of obesity in adults [5]. In a previous observational study, we reported a relationship between the diversity of the intestinal microbiota and weight gain in VLBW infants [6]. Kitajima et al. suggested that supplementation with *Bifidobacterium breve* might improve gastrointestinal tolerance and weight gain in VLBW infants colonized with *B breve* [7]. *Bifidobacterium lactis* has been shown to evoke a similar effect in term infants [8]. Reports suggest that full enteral feeding might be achievable earlier in preterm infants who receive supplements of *Lactobacillus sporogenes* alone [9], a mixture of *Lactobacillus* GG and bovine lactoferrin [10], a combination of strains of *Bifidobacterium* species [11], or a combination of *Bifidobacterium* and *Lactobacillus acidophilus* [12]. Randomized, controlled trials that consider weight gain as the main outcome are scarce, and none of these studies showed an improvement in weight gain [2]. The probiotic supplements used in these studies included *Lactobacillus* [9,13] and *Saccharomyces boulardii* [14], but not *Bifidobacterium*.

The primary objective of this study was to evaluate the effect of *Bifidobacterium* supplementation on short-term postnatal growth and body composition in VLBW infants, and its secondary objective was to assess the safety of probiotic administration.

2. Subjects and methods

2.1. Study design

This was a multicenter, double-blind, randomized, placebo-controlled trial that compared two groups of patients treated with probiotics or a placebo.

2.2. Population

Preterm infants who were hospitalized at French tertiary care centers in Lyon, Montpellier, and Bron, were eligible to participate in the study if they met the following criteria: a gestational age (GA) at birth of between 25 weeks and 31 weeks, a birth weight of between 700 g and 1600 g that was appropriate for the GA according to Usher's reference growth curves, admission to a participating unit within seven days of life, enteral feeding initiated before the fifth day of life, and the receipt of written parental consent. Infants were not eligible to participate in the study if they presented with NEC at ≥stage 1B [15], a severe malformation or any gastrointestinal malformations, or a severe medical or surgical condition. Furthermore, infants were not eligible to participate if their mothers had not been administered antenatal steroids or if their parents lived at too great a distance from the participating center to attend the follow-up visits. Participating infants were subsequently excluded from the study if any of the following occurred: an interruption of enteral or oral feeding for more than 72 h caused by severe gastrointestinal disorders, including Bell's stage NEC ≥ 2A [15], major gastrointestinal surgery, a confirmed or suspected intolerance to cow's milk, prompting the use of protein-hydrolyzed formula, or the withdrawal of parental consent.

2.3. Interventions

The preterm infants received one capsule daily that contained either probiotics plus maltodextrin if they were in the probiotics group (group P), or maltodextrin alone if they were in the control group (group C). The intervention was blinded, because the powders within the capsules were similar in color and appearance. Three probiotic mixtures were used: group P1 received *B. lactis*, group P2 received *B. longum*, and group P3 received *B. lactis* and *B. longum*. The quantity of probiotics administered was 10^9 colony-forming units/d, which was similar to that used in the first randomized, controlled trial [16]. Each capsule contained 250 mg of powder, which was dissolved in 1 mL of sterile water at the bedside and was administered by nurses at the beginning of the midday feed. Quality control of the capsules was performed every six months during the study. Infants started to receive the supplement before the end of the first week of life, and they continued to receive the supplement for four weeks if their GA at birth was ≥29 weeks or for six weeks if their GA at birth was ≤28 weeks. Duration of intervention depended on GA because we aimed to evaluate probiotic supplementation during a minimal 4 weeks period, and 2 weeks more in younger babies in whom it is relevant to assess postnatal growth over a longer period.

The participants, care providers, and those assessing patient outcomes were blinded to the interventions administered.

2.4. Number of subjects

The sample size was calculated to permit the detection of a 150 g difference in body weight at the end of the intervention period between the placebo and the pooled probiotics groups, which is less than the 200 g difference in body weight reported by Kitajima et al. [7], with a randomization ratio of 1:3, a power of 90%, and an α-error of 5%. Assuming an early termination rate of 10%, we planned to include 50 subjects in group C and 150 subjects in group P. With an early termination rate of 20%, the sample size allowed the detection of a 180 g difference in body weight between the placebo and pooled probiotics groups, with a power of 90%.

2.5. Randomization and group allocation

Infants were assigned to their treatment groups according to a pre-established randomization list that was stratified according to the investigating center and the GA at birth (≤28 weeks or ≥29 weeks), with a block size of four. Two treatment groups were defined, the control group (group C) and the probiotics group (group P), with the latter being composed of three subgroups (P1, P2, and P3). Each patient was randomized to one of the four treatment groups (C, P1, P2, or P3) with a 1:1:1:1 ratio within each center and stratum, leading to a 1:3 randomization in relation to group C and group P, which formed the focus of the primary analysis. The 1:3 randomization ratio in favor of group P was justified by the known protective effect of probiotics against NEC in preterm infants [16]. The randomization sequence was generated by CK using PROC PLAN with SAS® version 9.1 (SAS Institute Inc., Cary, NC, USA). Patients were allocated to receive the different treatments using consecutively numbered, sealed, opaque envelopes for each center and stratum.