Probiotics supplementation and length of hospital stay in neonates with gastrointestinal surgery

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A B S T R A C T

Any manipulation on open bowel causes interventional impact on gut microbiome, and surgical stress triggers bacterial translocation; thus, it will be fundamental to determine gut microbiome after surgery. Monitoring dynamic changes in microbiome of post-surgical infants who received probiotics and placebo could provide with important information about gut colonization and potential bacterial overgrowth. The purpose of this study is to assess the effect of probiotics supplementation on length of hospital stay, duration of parenteral nutrition, and feed tolerance in neonates after gastrointestinal surgery.

Introduction and rationale

Intestinal microbiota and nutrition are the leading factors for postnatal gastrointestinal (GI) tract development. Acquisition of gut microflora is a complex process that starts immediately after birth and continues throughout early childhood [1]. In addition to host-related features, several extrinsic factors such as the bacterial load of the environment and antimicrobial therapy influence the formation of the GI microbiome. Exposure to “healthy” environmental flora in early childhood is essential to prevent inflammatory and autoimmune diseases later in life [2].

Congenital defects of gastrointestinal tract and acquired conditions such as necrotizing enterocolitis (NEC) commonly require surgical intervention in the neonatal period. Intestinal surgery during this critical period of microbiome acquisition results in aberrant colonization of the GI tract by several pathways [3,4]. Surgical stress is known to cause disruption of the gut barrier and increase intestinal permeability and bacterial translocation [4]. This process triggers exaggerated immune responses that lead to inflammation and sometimes infectious complications [5,6]. Post-operative use of antibiotics have been shown to potentiate growth of pathogenic bacterial species such as Proteobacteria in favor of common microorganisms, Bifidobacteria and Lactobacilli, seen in the GI tract of healthy neonates and infants [7]. Delayed enteral feeding and prolonged use of parenteral nutrition in neonates with gastrointestinal surgery have been reported to cause luminal nutrient deprivation and bacterial dysbiosis [1,3]. All these changes in the GI tract of neonates are known to increase the risk for feeding intolerance, prolonged use of parenteral nutrition, and post-operative infection, which are the main drivers for prolonged hospital stay in this population.

Probiotics are live microorganisms that provide an opportunity to balance the intestinal microbiota and prevent bacterial overgrowth, promote gut barrier function, and modulate the local immune response [7–9]. Probiotics can regulate bacterial overgrowth by increasing the concentration of beneficial microorganisms and antagonizing pathogenic bacteria [10]. Furthermore,
probiotics have been described to improve gut motility via shortening phase-3 intervals in migrating motor complex [11]. Probiotics supplementation has been reported to increase villus height to crypt depth ratio in duodenum and ileum which results in a significant increase in intestinal absorptive area which largely helps digested nutrients pass into the villi [12]. These factors may explain the beneficial effects of probiotics on feeding tolerance of preterm infants [13].

Safety of probiotics was well confirmed in 26 Randomized Controlled Trials (RCT) in more than 6000 neonates [14–16]. No significant adverse effects from probiotic use were reported during routine use of probiotics in more than 3000 neonates [17,18]. There is no data on the toxic or lethal dose of probiotics for the preterm infant, and possibility of probiotic-induced infection is very low in this population [19]. Few reported cases of Lactobacillus septicemia were observed in chronic immunocompromised patients. Latest study on similar strains showed that Lactobacillus strengthen intestinal barrier function and tight junction integrity in experimental necrotizing enterocolitis (NEC) [20].

Probiotics have been started in the neonatal intensive care units (NICU) in Calgary to decrease the incidence of NEC. Currently approved for neonatal use by Health Canada probiotics strains are FloraBabyTM (Renew Life Canada, Oakville, Ontario, Canada) [21]. Each sachet (1 g) has Probiotic Blend 4 Billion Colony-forming Units (CFU), containing of Bifidobacterium breve (HA-129) 1.2 Billion CFU, Lactobacillus rhamnosus (HA-111) 1 Billion CFU, Bifidobacterium bifidum (HA-132) 800 Million CFU, Lactobacillus longum subsp. infantis (HA-116) 600 Million CFU, and Bifidobacterium longum subsp. longum (HA-135).

At the present time, infants with a history of gastrointestinal surgery are excluded due to lack of data about the use of probiotics in neonates with gastrointestinal surgery. Randomized control studies in adults reported improved intestinal microbial population, significantly decreased rate of postoperative infection, and a shortened duration of hospital stay in patients undergoing colorectal surgery [16,17]. A recent systematic review and meta-analysis of 20 trials (N = 1374 participants) reporting postoperative infections in adults with abdominal surgery reported a significant reduction in surgical site infection, urinary tract infection, and combined infections with no difference between groups for adverse events or mortality [8]. Little is known about using probiotics in neonates with intestinal surgery. Ezaki et al. reported a lower incidence of cow’s milk protein allergy and lower C-reactive protein levels in 18 neonates received probiotics after intestinal surgery [6].

Indications for gastrointestinal surgery in a first week of life could vary in infants of different gestation at birth. Knowing that any manipulation on open bowel causes interventional impact on gut microbiome, and surgical stress triggers bacterial translocation, it will be fundamental to determine gut microbiome after surgery. Monitoring dynamic changes in microbiome of post-surgical infants who received probiotics and placebo could provide with important information about gut colonization and potential bacterial overgrowth.

**Research objectives**

The main objective of this study is to determine the impact of probiotics administration on length of hospital stay in neonate undergoing gastrointestinal surgery.

**Primary outcome**

Length of hospital stay in neonates after gastrointestinal surgery.

**Secondary outcomes**

1. Duration of parenteral nutrition
2. Time to reach full feed (defined as 120 ml/kg/day) in the postoperative period
3. Incidence of infection as defined by positive bacterial blood, urine or cerebrospinal fluid culture
4. Incidence of cholestasis
5. Duration of cholestasis
6. Growth anthropometrics (Weight, length and head circumference Z scores)
7. Diversity and abundance of stool microbiome at baseline and after 1 and 3 weeks of initiation of probiotics

**Relevance**

Our project has the potential to promote the health of Alberta’s children and their families. Shortening the length of hospital stay, improvement of feed tolerance, prevention of complications of parenteral nutrition in neonates with gastrointestinal surgery are potential health benefits for our project.

**Methodology**

**Study design**

This study will be a randomized controlled trial NICU at Alberta Children’s Hospital (ACH) and Stollery Children’s Hospital (SCH). Probiotics or placebo will be administered orally or via naso- or orogastric feeding tube. Clinical outcomes and gut microbiome data will be compared between the two groups. The project starting time is January 2018. Study duration will be 18 months.

**Study population**

Infants admitted to NICU at Alberta Children’s Hospital and Stollery Children’s Hospital for gastrointestinal surgery on open bowel.

**Inclusion criteria**

1. Infants admitted to NICU ACH and SCH.
2. Required gastrointestinal surgery in a first week of life (including spontaneous intestinal perforation, necrotizing enterocolitis, bowel atresia, mechanical bowel obstruction, volvulus, gastrochisis)
3. Ready to start enteral feeding

**Exclusion criteria**

1. Infants with major congenital anomalies excluding GI tract
2. Suspected congenital or acquired immune deficiency
3. Palliative care patient
4. Septic babies with positive blood, CSF or urine culture

**Study aim**

The purpose of this study is to assess the effect of probiotics supplementation on length of hospital stay, duration of parenteral nutrition, and feed tolerance in neonates after gastrointestinal surgery. The study will also generate important data about safety and efficacy of probiotics in this surgical population.
Sample size and feasibility

A study by Dang et al. revealed a difference of 3 days in the time to reach full feeds in non-surgical preterm infants receiving probiotics compared to those in the placebo group [3]. We hypothesize that similar difference will be observed in the length of hospital stay between probiotics and placebo groups. A sample size of 44 infants per group will allow for a power of 0.8 and an alpha of 0.05 for a two-tailed detect similar difference in surgical neonates. Assuming that 70% of parents’ consent to their infant’s participation in the study, it will be feasible to complete enrollment of 88 babies in the 2 year period of the study.

Variables collected

a) Demographic variables: Gestational age, birth weight, gender, mode of delivery, singleton/multiple and rank, maternal administration of corticosteroids and antibiotics, duration of rupture of membranes, chorioamnionitis.

b) Clinical variables: Type of surgery, day of life at start of feeds post-surgery, total days of study drug, antibiotic use pre-and post-surgery, type of feeds during NICU and at discharge.

c) Outcome variables: mortality, time taken to reach full enteral feeds before (if applicable) and after surgery (defined by 120 ml/kg/day), post-operative total days of NPO and TPN, duration of central catheter use, number of separate NPO events, head circumference and weight at discharge home, post-operative infection (blood, urine or CSF), length of hospital stay, and incidence of cholestasis (defined by conjugated bilirubin >34 micromol/L).

Study protocol

Randomization

Infants will be identified within 48 h of surgery and parents will be approached for informed consent. Once consent is obtained, subjects will be randomly assigned to receive either probiotics or placebo. Investigators will conduct the randomization using a computer-generated table of random numbers generated at the University of Calgary.

Preparation and administration of study drug

FloraBabyTM that will be used in the study are the only probiotic agents in post-surgical infants. There is no data suggestive to discourage the appropriate use of the probiotic agents in post-surgical infants.

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Library preparation and sequencing

Sequencing of the V3-V4 region of the 16S rRNA gene will be performed on all cases and control samples. We will aim to recruit no more than 50% of our cases from VRE positive patients. Preparation of 16S metagenomics libraries and deep sequencing will be carried out by the University of Calgary’s Core DNA Services. DNA libraries will be prepared using the 16S Metagenomic sample preparation protocol (Illumina, San Diego, CA). The quality of the prepared library will be checked using Agilent TapeStation D1000 screen tape (Agilent Technologies, Santa Clara, CA), according to the manufacturer’s instructions.

Indexed DNA libraries are normalized to 4 nM and Illumina Experiment Manager used to build library plates and create sample sheet. Paired-end 300 bp sequencing will be performed on the MiSeq instrument using the V3 600 cycle MiSeq cartridge and MiSeq v3 reagents. The completed run will be demultiplexed with Illumina’s Casava software and stored in BaseSpace (Illumina) for downstream analysis.

Bioinformatic analysis

Sequences will be processed with the UPARSE pipeline as implemented in USEARCH v 8.0.1623 [20]. Details for each processing step are already established and would be performed similarly to previous studies done at University of Calgary.

Anticipated results and impact of project

We expect a decrease in the length of hospital stay after introduction of probiotics in infants with gastrointestinal surgery. We also anticipate the following positive outcomes from this study:

1) Decrease of duration of parenteral nutrition of infants in with gastrointestinal surgery
2) Reduction in parenteral nutrition associated liver disease
3) Decrease in the rate of post-operative and central line associated bloodstream infections
4) Generate data on microbial communities in neonates with gastrointestinal surgery.

Integrated knowledge sharing/translation plan

Our study team anticipates that the project will produce evidence related to potential benefits of using probiotics neonates with gastrointestinal surgery. This will help inform the decision to use or not to use probiotics in this population given the multi-disciplinary approach of the study and the involvement of investigators in the decision making of using probiotics in neonates. While there are local implications for the use of probiotics in surgical neonate infants in Calgary, it is clear that the study findings will have strong potential to be transferable to other neonatal intensive care in Alberta and the rest of Canada.

Integrated knowledge translation for our project is reinforced by the following processes for leading the implementation and sustainability of change:

1) Neonatology Medical lead of the surgical neonatal intensive care unit is engaged early in this research question;
2) A Neonatologist, a gastroenterologist and an infection control physician who are involved in the introduction of probiotics for non-surgical neonates are also involved in the study; and
3) Nutrition and pharmacy Services are collaborators.

Guidelines for using probiotics in neonates with gastrointestinal surgery would be developed based on the research results and disseminated through Neonatology and Nutrition pharmacy services. Results for the study will be presented in national and international conferences (Pediatric Academic Societies (PAS), American Academy of Pediatrics (AAP), Canadian National Perinatal Research Meeting (CNPRM) and conferences of Union of European Neonatal & Perinatal Societies (UENPS).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.isjp.2017.10.001.

References

[1] S. Fanaro, R. Chierici, P. Guerrini, V. Vigi, Intestinal microflora in early infancy: composition and development, Acta Paediatr. 91 (441) (2003) 48–53.
[2] F. Guarner, J.R. Malagelada, Gut flora in health and disease, Lancet 361 (9356) (2003) 512–519.
[3] R. Mackier, A. Sghir, H. Gaskins, Developmental microbial ecology of the neonatal gastrointestinal tract, Am. J. Clin. Nutr. 69 (Suppl.) (1999) 1035–1045.
[4] S. Bengmark, Ecological control of the gastrointestinal tract. The role of probiotic flora, Gut 42 (1) (1998) 2–7.
[5] S. Ezaki, K. Itoh, T. Kunikata, et al., Prophylactic probiotics reduce cow’s milk protein intolerance in neonates after small intestine surgery and antibiotic treatment presenting symptoms that mimics postoperative infection, Allergol. Int. 61 (1) (2012) 107–113.
[6] L. Lundell, Use of probiotics in abdominal surgery, Dig. Dis. 29 (6) (2011) 570–573.
[7] Y. Kawase, T. Ishii, H. Arai, N. Uga, Gastrointestinal perforation in very low-birthweight infants, Pediatr. Int. 48 (2006) 599–603.
[8] P. Stark, A. Lee, The bacterial colonization of the large bowel of pre-term low birth weight neonates, J. Hyg. 89 (1982) 59–67.
[9] F. Fouhy, C.M. Guinane, S. Hussey, et al., High-throughput sequencing reveals the incomplete, short-term recovery of infant gut microbiota following parenteral antibiotic treatment with ampicillin and gentamicin, Antimicrob. Agents Chemother. 56 (11) (2012) 5811–5820.
[10] B. Deplancke, O. Vidal, D. Ganesser, et al., Selective growth of mucolytic bacteria including Clostridium perfringens in a neonatal piglet model of total parenteral nutrition, Am. J. Clin. Nutr. 76 (5) (2002) 1117–1125.
[11] E.A. Deitch, Role of bacterial translocation in necrotizing enterocolitis, Acta Paediatr. Suppl. 396 (1994) 33–36.
[12] G.A. Nieuwenhuijzen, E.A. Deitch, R.J. Goris, Infection, the gut and the development of the multiple organ dysfunction syndrome, Eur. J. Surg. 162 (4) (1996) 259–273.
[13] D.A. Goldmann, J. Leclair, A. Macone, Bacterial colonization of neonates admitted to an intensive care environment, J. Pediatr. 93 (1978) 288–293.
[14] V. Nair, A. Soraiah, Probiotics and prebiotics: role in prevention, of nosocomial sepsis in preterm infants, International Journal of Pediatrics 2013 (2013) 1–8.
[15] K. AlFalah, J. Anabrees, Probiotics for prevention of necrotizing enterocolitis, Cochrane Database Syst. Rev. 4 (2014) CD005496.
[16] T. Fujii, Y. Ohtsuka, T. Lee, et al., Bifidobacterium breve enhances transforming growth factor beta1 signaling by regulating Smad7 expression in preterm infants, J. Pediatr. Gastroenterol. Nutr. 43 (2006) 83–88.
[17] H. Kita, J. Minami, M. Tanaka, et al., Early administration of Bifidobacterium breve to preterm infants: randomized controlled trial, Arch. Dis. Child. Fetal Neonatal Ed. 76 (1997) F101–F107.
[18] C. Deshpande, S. Rao, S. Patel, Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials. Lancet 2007 (369) (2007) 1614–1620.
[19] A. Trivedi, W. Chan, E. Teo, W. Turnow-Mordi, Probiotics for the post-operative management of term neonates after gastrointestinal surgery, Cochrane Database of systematic reviews. 2016, Available from: http://www.cochrane.org/CD0012265/NEONATAL-probiotics-post-operative-management-term-neonates-after-gastrointestinal-surgery.
[20] B. Blackwood, C. Yuan, D. Wood, et al., Probiotic Lactobacillus species strengthen intestinal barrier function and tight junction integrity in experimental necrotizing enterocolitis, J. Probiotics Health 5 (1) (2017) 159.
[21] Licensed Natural Health Product Database Data Extract, Available from: https://www.canada.ca/en/health-canada/services/drugs-health-products/natural-nondescription/applications-submissions/product-licensing/licensed-natural-health-product-database-data-extract.html.