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

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ABSTRACT

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,

Abbreviations: CFU, Colony-forming Units; EBM, Expressed Breast Milk; GI, gastrointestinal; GA, gestational age; NICU, neonatal intensive care units; NEC, necrotizing enterocolitis; RCT, randomized controlled trials.

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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, Bifidobacterium 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.

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.

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 post-operative 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 abundancy 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' 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, gastroschisis)
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

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 probiotics preparation recommended for use in neonates by Health Canada. Dose and route of administration are approved by Health Canada No Objection Letter (NOL) 80020959.

The study supplementation will be started at the when oral feeds will reach 24 mL/kg/day after surgery after collecting a stool/ostomy sample. The decision to start feeding will be made by the neonatal and surgical team. After the first stool/ostomy sample is obtained, one study sachet to a minimum of 1 mL of Expressed Breast Milk (EBM) (mothers own milk or donor human milk) once a day. Placebo and probiotics sachet will look the same, with no identifiers visible to care providers.

If mothers own milk or donor human milk is not available, 1 sachet will be added to a minimum of 1 mL of sterile water or formula (in formula fed babies) once a day. All empty sachets will be placed into biohazard container. Placebo sachet will be made of 0.3 g maltodextrin and be administered to the control group in the same manner. If the infant is placed NPO, the study drug will be stopped and restarted together with refeeding. Both probiotic and placebo will be packaged as single dose sachet. They will be maintained, blinded and dispensed by the pharmacists at the study sites, who will be the only ones knowing the subject assignment.

The study probiotic/placebo will be given till discharge. If the infant is transferred outside ACH NICU, the study drug will be dispensed with the infant and clinical outcomes will be ascertained in collaboration with the local Pediatrician at the time of infants' discharge home.

Safety monitoring

Probiotic research came from basic science and cohort studies to conclusive meta-analysis and long-term follow-up. Infections associated with probiotics use are extremely rare; NICUs of Japan, Italy and Finland have been using probiotics on regular bases at least for two decades, and have not reported any significant adverse reaction [20]. However, this possibility of severe compilations could not be ignored in certain populations. There are few reported cases describing Lactobacillus sepsis after use of this probiotics, however detailed review showed that these complications occurred in chronic patients with multiple medical conditions. Possibility of underlying immunodeficiency was never excluded. There is no data suggestive to discourage the appropriate use of the probiotic agents in post-surgical infants.

Inclusion and exclusion criteria for this study were made to ensure the safety of infants. Chronic patients or infants with multiple congenital anomalies will not be enrolled in the study as well as infants who are at high risk to develop short bowel syndrome. Independent Safety Committee consisting of three physicians will be created to assure and review safety of probiotics use in this population. First review will be done after first 30 patients are enrolled. Study will be stopped if more than one of the enrolled subjects develops infection from any of probiotics strain. Furthermore, infants who are at high risk to develop short bowel syndrome (remaining bowel less than 50% of the expected intestinal length) are excluded.

Sample collection

Nurses will collect the stool samples at 3 time points: prior to initiation, 1 week after and 4 weeks after probiotic or placebo administration is commenced. "Stool" will be collected directly from the infant's ostomy bag and diaper with a sterile spatula. As soon as a sample is collected, the nurse will contact the investigators. The samples will then be placed in a laboratory freezer (−80 °C) within 24 h of collection. Batched samples from both sites (Calgary and Edmonton) will be transported to the University of Calgary Genomics laboratory for subsequent microbiome processing.

Microbiome DNA extraction, amplification and species identification

DNA extraction

DNA will be extracted from ~200 mg of frozen (−80 °C) stool using the Zymo ZR Fecal DNA Stool MiniPrep Kit (Zymo Research Corp, USA).

Real-time quantitative PCR (qPCR)

Quantification of target bacterial DNA will be measured using the SsoFast™ EvaGreen® Supermix (Biorad, Mississauga, Canada). Samples and standards (10 ng of DNA from known bacteria) will be prepared and run in duplicate in a final volume of 20l on 96 well plates using the CFX96 detection system (Biorad, Mississauga, Canada). All collected fecal samples will be quantified for *Bacteroides*, *Prevotella*, *Clostridium coccoides* and *C. leptum*, *Enterococcus* and *Enterobacteriacae* sp. as indicators of microbiome health [19].

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 that 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 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 woul 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 blood stream 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 multidisciplinary 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.

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