Impact of Killed Probiotics on Intestinal Immunity in Preterm Neonates

Thesis

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Introduction

Probiotics bacteria are live microbial supplements that colonize the gastrointestinal tract and potentially provide benefit to the host **(Millar et al., 2003).**

Probiotics may protect high risk infants by increasing the barrier to translocation of bacteria and bacterial products across mucosa, competitively excluding potential pathogens, modifying host response to microbial products and enhancing enteral nutrition that inhibits the growth of pathogens such as Klebsiella pneumonia, Escherichia coli and Candida albicans. So altering microbial flora by enteral feeding of probiotics might be beneficial **(millar et al., 2003) (Dani et al., 2002).**

VLBW preterm neonates are at risk of gut colonization with pathogens which can alter the permeability of the intestines and promotes inflammatory cascade **(Gewolb et al., 1999).**

Secretory immunoglobulin A (SIgA), the most important and predominant immunoglobulin in mucosal surface, provides protection against antigens, potential pathogens, toxins, and virulence factors **(Forchielli and Walker, 2005).** The development of IgA producing plasmablasts in the intestinal mucosa, precursors for sIgA, is influenced greatly by the micro flora **(Cebra, 1999).**

Enteral delivery of live probiotics to patients at higher risk of sepsis could cause secondary nosocomial infections in these individuals, because gut mucosal barrier is impaired during sepsis **(De Groote et al., 2005).**

Oral feeding heat -killed probiotics resulted in modulation of host immune function, leading to a stable and safe preparation without alteration of clinical efficacy **(Gill et al., 2001) (Xiao et al., 2003).**

Aim of the Work

The aim of this study was to evaluate the effect of killed probiotics on intestinal immunity of preterm neonates by measuring secretory IgA in stools.

Probiotics

The term probiotic was introduced into the scientific literature in the 1965 by Stillwell and Lilly. A widely accepted definition of probiotics is 'live microbial food ingredients that are beneficial to health However, the scientific basis of this definition has recently been questioned since animal studies suggest that some probiotic effect can be achieved by nonviable bacteria and even by isolated bacterial DNA. Therefore, probiotics have more been recently defined as 'microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well being of the host **(Salminen, 1999).**

There are several generally accepted characteristics that define probiotic bacteria **(Saavedra et al., 2001).**

Probiotics

- Are microbial organisms
- Remain viable and stable after culture, manipulation, and storage before consumption
- Survive gastric, biliary, and pancreatic digestion
- Are able to induce a host response once they enter the intestinal microbial ecosystem (by adhering to gut epithelium or other mechanisms); and

- Yield a functional or clinical benefit to the host when consumed.

Breast milk, Formula and Intestinal Colonization:

Breast fed infants have higher concentrations of protective Bifidobacteria and Lactobacillus in their gastrointestinal tract, whereas Streptococcus faecalis, Staphylococcus, E.coli and Clostridia are in higher levels in formula-fed infants **(Agostoni et al., 2004).**

Premature infants who receive breast milk have fewer episodes of late-onset sepsis, NEC, diarrhea and urinary tract infections, and are less likely to require antibiotic therapy **(Manzoni, 2007).** Evidence suggests that a lack of breastfeeding a long with increase antibiotic use have separate but interactive negative effects on gut flora diversity **(Gewolb et al., 1999).** Human breast milk also contains three components that inhibit colonization by harmful bacteria (1) Secretory IgA, which is produced by the maternal immune system against enteric pathogen that had previous exposure **(New-burg, 2000).** (2) Fatty acid that destroy viruses and act against other pathogens when released from the triglycerides of milk in the stomach of the infants; and (3) lactoferrin, the major protein in human milk, which is active against a broad spectrum of pathogenic bacteria **(New-burg, 2000).**

Consequences of antibiotics in NICU infants

The NICU population is routinely exposed to antibiotic therapy. Premature infants often receive antibiotic as early as the first day of life. Some newborns are on prophylactic oral antibiotics for hydronephrosis or recurrent urinary tract infections. Antibiotics alter the gut flora and can result in diarrhea or constipation, as well as foster the development of thrush or cutaneous candidal infections **(Saaverda, 2001).**

Sick newborn and premature infants treated with antibiotics are at risk for colonization with aggressive and drug-resistant bacteria, which may pose a greater risk of NEC and its complications **(Hoyos, 1999).** Infants who have been on antibiotic therapy for longer periods of time were noted to have the fewest number of different bacteria present in their intestinal system **(Gewolb, 1999).** A decrease in the number of normal flora is believed to be a risk factor for NEC. A probiotic given with the prescribed antibiotic theoretically may reduce the effect of microbial alteration and any adverse effects on stool consistency and frequency. Although a few studies have been conducted regarding prevention of antibiotic-associated diarrhea through the use of probiotics in older patients **(Jirapinyo, 2002),** studies in the neonatal population are currently lacking.

Clinical Benefits Reported With Probiotic

Acute diarrhea:

The best-studied clinical outcome with use of probiotic bacteria has been that of acute diarreal disease. The larger number of trials documents therapeutic use of probiotics as supplements early in the course of the disease. The majority of studies have included various species of lactobacilli, and far the most used has been Lactobacillus rhamnosus (GG). Four meta analyses have recently been reviewed **(Szajewska et al., 2006).** L. rhamnosus (GG), in particular, has shown efficacy when given as a supplement early in the course of Rota viral diarrhea. The most consistent effect reported is a reduction in duration of illness (by ½to 1½ days), while for the individual infant the effect may be modest, the larger effect on the population may be significant. Cost-benefit analyses and compliance assessment have not been done.

Another body of literature examines the reduction in incidence (prevention) of acute diarrheal disease. Several studies, with various levels of significance, document a reduction in incidence or severity of acute diarrhea with Bifidobacteria, mainly B. lactis, **(Chouraqui et al.,2004)**, and with Lactobacilli, mainly L. rhamnosus (GG), **(Szajewska et al., 2001),** though protection is not always significant **(Mastretta et al., 2002).** In addition, both L. rhamnosus (GG) and L. reuteri (during treatment) **(Rosenfeldt et al., 2002)**, and

Bifidobacterium lactis (used prophylactically) **(Saavedra et al., 1994),** have documented reduced rotaviral shedding.

A recent meta-analysis reviewed 34 randomized, clinical trials that evaluated the efficacy of probiotics in the prevention of acute diarrhea. Probiotics significantly reduced the risk of diarrhea developing in infants and children by 57% (confidence interval, 35%–71%). The protective effect did not significantly vary among the probiotic strains used, including Bifidobacterium lactis, Lactobacillus rhamnosus (GG), Lactobacillus acidophilus, Saccharomyces boulardii, and other agents used alone or in combination with 2 or more strains **(Sazawal et al., 2006).** These findings, in addition to reduced duration of hospitalization **(Isolauri et al., 1991)**, and decreased hospitalization, **(Guandalini et al., 2000)**; all suggest that the effect occurs on both the manifestations of the disease and on the course of the infection. No study to date has documented an increase (significant or not) in diarrheal disease with probiotic use. These observations greatly bolster the arguments for finding ways to use specific probiotics in a long-term and prophylactic manner, particularly in infancy.

Antibiotic associated diarrhea:

Several probiotic bacteria appear to be valuable in reducing the risk of antibiotic-associated diarrhea in infants and children **(Arvola et al., 1999)**.

Several probiotic have been used in an attempt to prevent antibiotic-associated diarrhea, Lactobacillus, Bifidobacteria, and streptococcus. However only Saccharromyces boulardii, Enterococcus faecium and Lactobacillus have been shown to be clinically effective in preventing antibiotic-associated diarrhea **(Rolfe, 2000).**

Allergy:

Microbial-gut interactions can improve the integrity of the gut barrier by decreasing intestinal permeability, reducing both adherence of potential antigens and their systemic effect, and by modulating GALT immune response toward antigen tolerance **(Kalliomaki et al., 2003)**. Lower counts of Bifidobacteria have been reported in atopic vs non-atopic children preceding allergen sensitization. Bifidobacteria are hypothesized to more effectively promote tolerance to nonbacterial antigens, primarily by inhibiting the development of a Th2-type (proallergic) response. Infants with atopic dermatitis who received hydrolyzed whey formula supplemented with L rhamnosus (GG) showed greater clinical improvement than those who received the hydrolyzed formula alone. They also excreted less TNF-α and α-1-antitrypsin in their stool, suggesting that the probiotics decreased gut inflammation. **(Isolauri et al., 1996).** Atopic infants treated with extensively hydrolyzed whey-based formula with L rhamnosus (GG) or B lactis showed greater improvement in severity of skin manifestations than with hydrolysate formula

alone. The probiotic-supplemented group also demonstrated a reduction in serum soluble CD4 (a marker of T-cell activation) and an increase in serum TGF-β1 (involved in suppressing the inflammatory response via IgA production and oral tolerance induction) **(Isolauri et al., 2000)**.

Studies suggest that regular probiotic supplementation may stabilize intestinal barrier function and play a role in modulating allergic responses leading to a decreased severity of atopic symptoms, particularly atopic dermatitis associated with cow's milk protein **(Pohjavuori et al., 2004).**

In a recent study, a positive change in stool colonization in atopic infants supplemented with B. lactis has been shown with a decrease of Bacteroides and E coli in the stool. Most interestingly, serum IgE correlated with E coli counts, and in highly sensitized infants, IgE correlated with Bacteroides counts. Thus, certain probiotics seem to influence the gut's allergen-stimulated inflammatory response and provide a barrier effect against antigens that might otherwise ultimately lead to systemic allergic symptoms (such as eczema) **(Kirjavainen et al., 2002).**

NEC:

The newborn gut microflora foster integrity of the immune system protects from infections with enteric pathogens, produce vitamins, and encourage mucosal maturation **(Magne et al., 2005).** The colon of the preterm infants is sterile at birth,

but is rapidly colonized soon after birth **(Dani et al., 2002).** Identified microorganisms that colonize the preterm newborn intestine include manily coliforms, enterococci and bacteroides species **(Bin-Nun et al., 2005).** Bifidobacteria commonly found in the term newborn gut, are not detected in VLBW infants receiving breast milk during the first two weeks after birth. Bifidobacteria and Lactobacillus could be found in the stool of less than 5 percent of preterm infants within the first month of life **(Dani et al., 2002).**

The premature infant is exposed to a variety of factors that negatively affect their possibilities of attaining an adequate or appropriate colonization. These factors include increasing exposure to potential delayed colonization, colonization with "neonatal intensive care unit (NICU) environmental microbes", use of antibiotics, lack of exposure to normal maternal flora and breast milk, invasive procedures, immature mucosa, and increased chances for bacterial and antigenic translocation. The combination of an increase in potentially pathogenic microorganisms together with a decrease in normal flora found in preterm neonates is speculated to be one of the factors that make these infants more at risk for overgrowth of potentially pathogenic species and the development of NEC **(Bin-Nun et al., 2005).** Cases of NEC cluster in time and place; germ-free animals do not get NEC, and changes in bacterial metabolic activity (hydrogen gas production) precede the development of NEC **(Magne et al., 2005).** These observations strongly support

the idea that microflora establishment and composition in premature infants is a major determinant in the pathophysiology of NEC. The theoretical benefits of probiotics in preterm infants include reduction of enteric pathogens, improved gut structure and function, facilitation of enteral nutrition, reduced dependence on parenteral nutrition (PN), increased gut mucosal barrier function, reduction in sepsis and antibiotic use, and ultimately prevention of NEC **(Magne et al., 2005).**

Mechanisms by which probiotics could prevent NEC include increase in favorable type microflora with reduced colonization by pathogens, increased intestinal barrier to translocation of bacteria into the bloodstream, modification of the host response to microbial products by sensitization and immunization, and enhanced tolerance and advancement of enteral nutrition **(Agostoni et al., 2004).**

Specific bifidobacteria and lactobacilli, when given orally, are successful in transiently colonizing the gut of infants and young children. Significant increases in fecal Bifidobacteria have been reported as early as 1 week after supplementation. In some cases, these counts can reach levels similar to those found in breastfed infants **(Kullen et al., 2005).** Bifidobacteria supplementation in premature infants has also been shown to positively modify the microflora of the infants'intestine **(Mohan et al., 2006).** Beneficial increases in stool short-chain fatty acids, reduction in stool pH, and decrease in fecal ammonia, indoles **(Baker-Zierikzee et al., 2005),** and

concentrations of Bacteroides and E coli have been documented with Bifidobacteria supplementation **(Kirjavainen et al., 2002)**. As a result, specific probiotic bacteria positively affect the ratio of favorable to unfavorable microflora in infants and children and lead to positive changes in gut luminal environment.

Types of probiotics:

Lactic acid, or Lactobacillus, is among the most important probiotic microorganisms associated with the human gastrointestinal tract **(Dash, 2005).** Examples of specific probiotic organism important for infants include Lactobacillus acidophilus, Lactobacillus casei, and Lactobacillus rhamnosus (also called Lactobacillus GG) Bifidobacterium lactis Bifidobacterium bifidum and bifidobacteria infantis. Lactobacillus acidophilus has been shown to prevent infectious diseases and favorably alter the intestinal microflora balance, thereby inhibiting the growth of harmful bacteria promoting good digestion, boosting immune function and increasing resistance to infection. Lactobacillus casei increases levels of circulating IgA in infants infected with rotavirus, thereby shortening the duration of diarrhea. Lactobacillus rhamnosus (Lactobacillus GG) has been effective in management of acute pediatric diarrheal disease **(Saavedra, 2001),** and reduces candida colonization in neonates **(Manzoni, 2007).**

Lactobacillus GG has also been associated with reduced atopic dermatitis in infants when administered to pregnant woman prenataly and during the first six months of the infant's life **(Michail et al., 2006).** Bifidobacterium lactis reduce the incidence of antibiotic-associated diarrhea in infants **(Correa et al., 2005).**

Bifidobacterium bifidum strengthens gastrointestinal immunity especially in children. Prophylactic administration of bifidobacteria infantis and Lactobacillus acidophilus was shown to reduce both the incidence and severity of NEC in premature neonates in one study **(Lin et al., 2005).**

How probiotics work

In order to be effective, probiotics must survive the acidic stomach environment and the alkaline condition of the duodenum **(Boyle et al., 2006).** Probiotic must also adhere to the intestinal mucosa of the colon. In adhering to the colonic mucosa, probiotics prevent the attachment of pathogenic bacteria **(Boyle et al., 2006).** Some probiotics increase the numbers of IgA and other immunoglobulin-secreting cells in the intestinal mucosa as well as stimulate the release of interferon **(Gorbach, 2000).** Other probiotics facilitate antigen transport to underlying lymph cells **(Singhi & Baranwal, 2008),** or reduce the ability of other pathogenic organisms to colonize the intestine either by producing acids that decrease the environmental pH or by secreting specific antibacterial