The role of probiotics in the prevention of bacterial infections and Candida in Neonatal Intensive Care. Prospective study with control groups

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Abstract

Introduction

The surface of the intestines of an adult human has an area of 200-250 m^2 that contains more lymphoid material in the intraepithelial lymphocytes, lamina propria, Peyer's patches and lymphoid follicles than all the rest of the body, and at the same time harbors bacterial flora composed of more than 400 species, subdivided into 17 families and 45 genera (1).
Over the course of evolution, a symbiotic balance has been created between these two systems, leading to the conclusion that the microbiology of the intestines forms an integral part of the host organism, i.e. a "bacterial organ" (2).
The composition of the intestinal microflora, crucial for the development of the immune system, depends on the method and location of childbirth.
Hospital Caesarean births show an elevated component of anaerobic microflora (Clostridia) and an increased load of gram negative enterobacteria.
On the other hand, vaginal births and early breastfeeding produce a microflora rich in Lactobacilli and Bifidobacteria.
Premature newborns admitted to Neonatal Intensive Care harbor a bacterial flora that is greatly modified, composed of a little less than 20 species, the predominant aerobes being Staphylococci (negative coagulate and aureus), Enterobacteria (Klebsiella) and Enterococci, the predominant anaerobes being Clostridia (3.4).
It is believed that microbiological diversity is an important factor in establishing the stability of the intestinal ecosystem and that the loss of such diversity predisposes premature infants to gastrointestinal colonization by fungi and antibiotic-resistant bacteria, resulting in the potential risk of infections (5).
Numerous studies conducted on experimental animals have in fact identified the diverse activities of intestinal microflora: anatomical-functional (trophism, maturation and motility of the intestines), protective (resistance to colonization by pathological strains), metabolic (absorption of nutrients and minerals, increased tolerance of lactose, production of short-chain fatty acids, reduction of cholesterol levels) and immunomodulating.
Immunological imprinting takes place in the first days of life when, in the newborn, there is a physiological prevalence of the Th2 cytokines, compatible with an inflammatory-allergic condition. Some intestinal bacteria, the Lactobacilli and Bifidobacteria in particular, modulate the immune response in favor of a Th1 profile, counter-regulating the associated IgE response and stimulating the production of secretory IgA (6, 7).
Therefore, post-natal microbiological imprinting conditions the first phases of the development of the immune system and it is subsequently controlled by a homeostatic mechanism (8).
Antibiotic therapy and infective events induce rapid changes in the intestinal microflora, while hygienic conditions and nutrition induce slow changes.
The direct introduction of particular microbiological strains into the diet, in particular, designated as "probiotics", can positively influence the intestinal microbial population (5).

To be defined as a probiotic, a microorganism must correspond to the following characteristics (9):

- Be of human origin
- Exert a beneficial effect on the host organism
- Be neither pathogenic nor toxic
- Have no transferable resistance to antibiotics
- Be capable of surviving gastrointestinal transit
- Have the ability to colonize the intestines
- Have a pleasant taste
- Possess clinical evidence of safety
- Have a favorable cost/efficacy ratio
- Remain alive during storage and use.

This last aspect is a determining factor in the efficacy of probiotics.
Numerous bacteria have been defined as probiotics, but only some present all the characteristics listed above, specifically the Lactobacilli, the Bifidobacteria and some Gram-positive cocci.
The functional interactions between probiotics on the one hand and the intestinal epithelium, the mucosal immune system and the systemic immune system on the other hand are at the foundation of the direct and indirect mechanisms of the effects of these bacteria.
The direct mechanisms are based on the concept of a direct competition between probiotics and pathogenic microorganisms, which explains the beneficial effects in the intestines, but does not explain more remote effects.
The indirect mechanisms also explain the extraintestinal effects, which consist essentially of an increase in the function of the intestinal barrier and in the modification of the immune response. The latter depends largely on the site of the interaction between the probiotic and the effectors of the immune response, topographically located in the intestinal tract (10).
There is evidence, in vivo and in vitro, of different effects of particular strains of probiotics on specific mechanisms of the immune response and of the fact that not all the probiotics have the same initial contact (immune cells, enterocytes, etc.). .
It is now known that such effects are strain-specific and not species - or genus - specific, and that the combined effect of more strains, common in the preparation of probiotic supplements, can contribute, depending on the specific case, both to a synergistic and an antagonistic action.
The target of certain probiotics, however, is determining for their immunomodulating effect, which may be predominantly local or be reflected in systemic or extrasystemic mechanisms.
In summary, the effects of probiotics are characterized by:

- stronger competition with pathogens for enteric nutrients and epithelial anchorage sites
- a reduction in intestinal pH values as a result of the increased production of lactic acid from lactose and acetic acid from carbohydrates, which favor the growth of Lactobacilli
- the production of antimicrobial substances, or bacteriocins, which are peptides possessing bactericidal activity towards corresponding bacteria
- the metabolization of any nutrients in volatile fatty acids
- the activation of mucosal immunity, with increased synthesis of secretory IgA and phagocytosis
- the stimulation of the production of cytokines (10).

The bacteria most frequently used as probiotics are the Bifidobacteria and certain bacilli, including *Lactobacillus rhamnosus* GG (LGG) and *Lactobacillus reuteri*.

LGG is the most studied probiotic; it exerts its antibacterial function in various ways:
- produces a wide spectrum of antibiocins, including pyroglutamate, which inhibits many bacteria without inhibiting other strains of Lactobacilli
- increases the bioavailability of antibiotics, reducing undesirable effects like diarrhea, distension, abdominal cramps, etc.
- attenuates and normalizes increased intestinal permeability

- stimulates the local release of cytokines and increases the number of cells that produce IgA in the intestinal mucosa
- stimulates the immunological barrier of the intestines
- reduces hypersensitivity reactions caused by food antigens.

Lactobacillus reuteri is considered one of the few species of autochthonous Lactobacilli in humans; it exerts its antibacterial function in various ways:
- converts glycerol into **reuterin**, a potent, wide-spectrum antimicrobial substance synthesized by its growth, which has a potent activity against both pathogenic bacteria and yeasts and fungi like Candida albicans. Its antibacterial action does not inhibit normal microflora, however.
- tolerates low gastric and intestinal pH better than other Lactobacilli, resulting in optimal survival and colonization of the gastrointestinal tract.
- stimulates the release of particular cytokines and increases the number of cells that produce IgA in the intestinal mucosa
- modulates the immune response by means of the CD4 and T-helper cells in the ileum

In recent years in Neonatal Intensive Care, it has helped increase the survival rate of newborns of very low weight, which suffer increased risk of gastrointestinal colonization by bacteria and/or fungi.

Objectives

Considering that infection rates in Neonatal Intensive Care are 25-40% (11), 11% being of fungal origin (12), we wanted to study the effect of two different probiotics separately on the reduction of infective risk (bacterial and/or fungal) and also verify whether, in newborns that have contracted a Candida infection, the addition of the probiotic to the antifungal treatment would contribute to a more rapid eradication.
Our study was conducted on 184 newborns, all preterm with an average gestational age of 34.9 ± 2.6 weeks and a weight of 2262 ± 730 g, admitted in the last two years immediately after birth to Neonatal Intensive Care at the Azienda Ospedaliera Universitaria Policlinico di Catania [*University Hospital of Catania*].
Infants suffering from congenital malformations that required major surgical interventions (esophageal atresia, diaphragmatic hernia, intestinal malformations) were excluded from the study.

Methods

The newborns were subdivided into three groups: Group I of 67 newborns treated with *Lactobacillus reuteri*; Group II of 55 newborns treated with LGG; Group III of 62 newborns that did not consume probiotics.
The characteristics of the newborns were similar in the three groups in terms of birth weight, gestational age, sex, etc. (Table 1).

Table 1. Demographic characteristics of the newborns

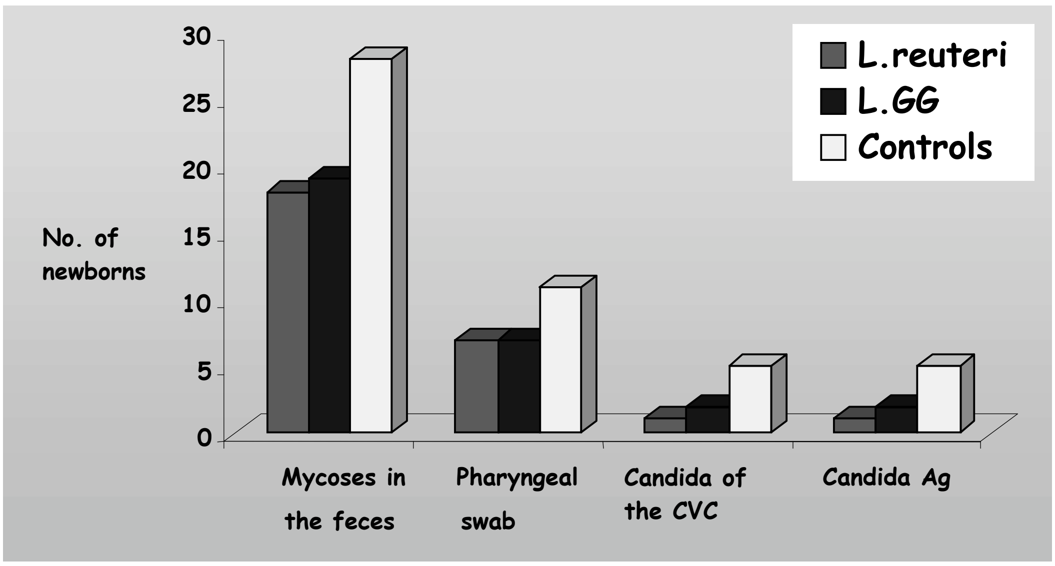
Parameters	*L. reuteri*	L.GG	Controls
No. of newborns	67	55	62
Gestational age (weeks)	34.7±2.9	35.3± 3.2	34.8±3.2
Birth weight (g)	2188± 718	2445± 664	2152± 696
Length (cm)	43.4± 7.5	44.6± 7.8	43.6± 7.5
Cranial circumference (cm)	30.5± 5.04	31.4 ± 5.1	30.7± 5.03
Male/Female	38/29	33/22	36/26
Caesarean sections	58/67	44/55	56/62
Assisted ventilation	24/67	26/55	22/62
Gestational age <32 weeks	14/67	7/55	6/62
Central venous catheter	37/63	27/55	28/62

Fecal cultures, gastric aspirations and pharyngeal swabs were performed at birth and at 7, 14, 21 and 28 days. The infants were considered at risk of systemic candidiasis if they presented 8 x 10^6 colony forming units (CFU) of Candida per g of feces. The clinical signs of the beginning of infection were monitored, including: fever, dehydration crisis, apnea, bradycardia, pallor or grayish coloring, necessity of O_2 supplementation and/or reintubation.
All the newborns were carefully examined at least twice daily for gastrointestinal symptoms such as regurgitation, vomiting, gastric retention, abdominal distension and characteristics of the feces. They were weighed daily, and the day on which parenteral nutritional was stopped was also recorded.
The following laboratory values were also monitored: organic liquid cultures, APC, blood count, Candida antigens. Tests for positive antigen levels were conducted by testing for the Candida mannan antigen using the Platelia Candida test.
Instrumental investigations were also conducted, including ultrasounds (renal, cardiac, abdominal, transfontanellar), examination of the fundus oculi and chest x-rays to detect any mycotic involvement of the organs.
Antimycotic treatment was conducted with liposomal Amphotericin B at an initial dose of 1 mg/kg/day with a gradual increase up to a maximum of 6 mg/kg/day in continuous infusion over 30 minutes. Treatment was stopped 7 days after a negative culture for the Candida antigen and 3 consecutive negative ATP tests.
The statistical analysis was conducted using the T-student test. Differences were considered significant at values of $p<0.05$.
Lactobacillus reuteri was administered at doses of 5 drops/day equivalent to 1 x 10^8 CFU, through a nasogastric tube or by mouth.
LGG was administered at doses of 1 capsule/day, equivalent to 3 x 10^9 CFU, after opening the capsule and dispersing it in water or milk, always administered through a nasogastric tube or by mouth.

Results

18 newborns in Group I, 19 of Group II and 28 in Group III tested positive for fungi in the feces.
7 newborns in Group I, 7 in Group II and 11 in Group III tested positive for Candida with the pharyngeal swab. The growth of Candida in the central venous catheter culture and antigen levels were positive in 1 newborn in Group I, in 2 newborns in Group II and in 5 in Group III (Graph 1).
The number of Candida infections was significantly higher ($p<0.05$) in the control group (6.4%) with respect to the group treated with *L. reuteri* (1.4%) and to that treated with LGG (3.6%). Also, the number of bacterial infections was significantly higher ($p< 0.05$) in the control group (9.6%) than in the group treated with *L. reuteri* (1.4%) and that treated with LGG (3.6%) (Table 2).

Graph 1. Parameters of Candida infection



Graph 2. Prevalence of gastrointestinal symptoms

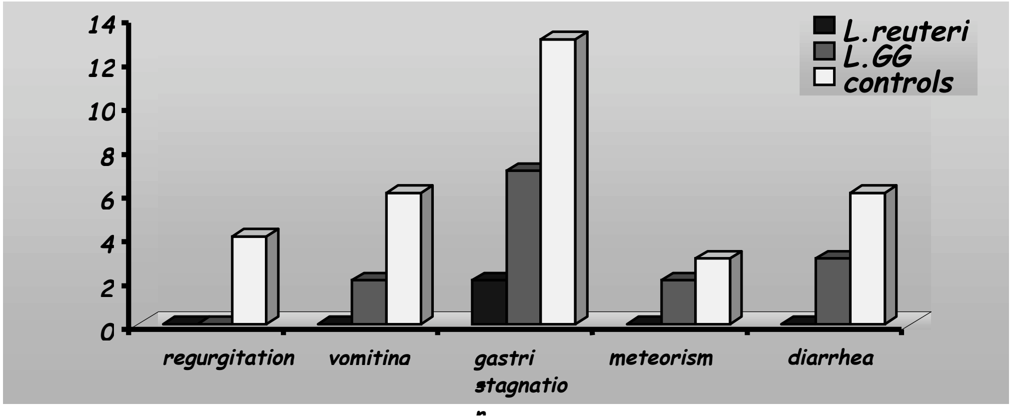


Table 2. Results

Parameters	L. reuteri (I)	L.GG (II)	Control (III)	p<0.05
No. of newborns	67	55	62	
Weight loss	107±33	128±72	155±20	I v III
Days of parenteral nutrition	6.6±6	10.7±9	13.9±13	I v III
No. of bacterial infections	1 (1.4%)	2 (3.6%)	6 (9.6%)	I v III II v III
No. of mycotic infections	1 (1.4%)	2 (3.6%)	4 (6.4%)	I v III II v III
Days of antimycotic treatment	16	21.7±2.6	40.7±16.2	I v III II v III
Days in hospital	21.9±16.9	28.6±19.3	42.3±28.9	I v III II v III

The days of parenteral nutrition were greater in the control group with respect to the groups treated with probiotics, even if the difference was statistically significant for Group I only.
The most frequent clinical symptoms were gastrointestinal symptoms (64%), fever (24%) and an increase in O_2 requirements (12%).
The group treated with *L. reuteri* presented a significant reduction ($p<0.05$) in gastrointestinal symptoms (2/67) with respect to the group with LGG (14/55) and the control group (27/62). (Graph 2)
In newborns with Candida infections, the supplementation of the antimycotic treatment with a probiotic treatment resulted in a significantly ($p<0.05$) more rapid eradication of the infection with fewer days of treatment in the two groups taking probiotics (18.8 ± 3) with respect to the control group (40.7±16.2).
The use of probiotics has enabled a significant reduction ($p<0.05$) in days of hospital stay.

Conclusions

Based on our preliminary data, the use of probiotics appears to be effective in the prevention of both bacterial and mycotic infections, in the containment of gastrointestinal symptoms and in a more rapid weaning from total parenteral nutrition with a reduction in the central venous catheter time and the number of days in hospital.

In the group of newborns treated with *L. reuteri*, we noted minor gastrointestinal symptoms, earlier achievement of tolerance for food by mouth, more rapid discontinuation of parenteral nutrition, less weight loss in the course of the first week of life and better growth (both height and weight) in the first month of life.

Also, in the group of newborns treated with *L. reuteri* and Amphotericin B, we saw a favorable evolution of the Candida infection and discharge after 16 days, compared to 35 days for the newborns in the control group.

The newborns treated with probiotics did not manifest involvement of organs.

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