Original Article

Effect of a lactose-free milk formula supplemented with bifidobacteria and streptococci on the recovery from acute diarrhoea

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Probiotics have been proposed for the management and prevention of acute diarrhoea in infants. A double-blind, randomised, placebo controlled study was carried out in 224 Chinese infants 6 to 36 months of age with severe acute diarrhoea and free from moderate or severe malnutrition. After oral or parenteral rehydration, they were allocated to one of three groups: a lactose-free formula (Control); the same formula but with viable 10^8CFU *B. lactis* Bb12 and $5x10^7$CFU *St. thermophilus* TH4 per gram of powder and, the same formula with the same microorganisms, but with 10^9CFU/g and $5x10^8$CFU, respectively. Anthropometric parameters, duration of the diarrhoea and rotavirus shedding were evaluated. Eighty seven percent of the episodes were associated with rotavirus infection. The duration of the diarrhoea was not influenced by the intake of probiotics. However, a decrease of rotavirus shedding was observed in infants fed the formula with 10^9 Bb12/g, a finding of probable epidemiological importance in the transmission of this agent.

Key Words: infants, acute diarrhoea, rotavirus, probiotics, *Bifidobacterium lactis* Bb12

INTRODUCTION

Despite considerable improvements in the management of acute diarrhoea resulting from better understanding of water and electrolyte balance and the importance of nutrition, considerable numbers of infants and children die every year or suffer negative consequences on their nutritional status.[1] Nutritional deterioration makes them more vulnerable to infections because of the associated derangements of immunity.[2]

That the resident colonic microbiota may be important in maintaining the health of individuals was postulated many years ago. The positive role of exogenous bacteria from fermented milk products was first suggested by Metchnikoff who proposed that lactobacilli present in yogurt promote health and prolong life.[3] Fuller coined the term probiotics to identify a group of microorganisms that are ingested live in food and would have positive effects on the host by improving his microbial balance.[4] As interest on these microorganisms grew it became logical to test them for their potential modulating effects on susceptibility to diarrhoea and its adverse nutritional consequences.[5] To influence gut physiology probiotics have to be ingested in sufficient numbers and to survive the effects of gastric acidity, bile salts and pancreatic enzymes. In addition, they must compete with the resident bacteria for receptors on the surface of enterocytes and eventually with pathogens, preventing their proliferation and adherence. Other desirable properties of probiotics are their capacity to stimulate the local and systemic immunity of the host and the phagocytic activity of neutrophils and macrophages.[6]

Of the many potential probiotic microorganisms, the majority are lactobacilli and bifidobacteria. The latter are most abundant in the colon of breastfed infants but their numbers decrease after weaning, ranging around 10^9 cells/g of stools. At this stage, they become subdominant compared to *Bacteroides* and *Clostridium*, which are 10 to 100 times more numerous.[7] Probiotics administered orally are capable of transiting along the gastrointestinal tract and can be detected in the faeces.[8] These microorganisms, including bifidobacteria have shown favourable effects on the evolution of infant diarrhoea, particularly that caused by rotavirus.[9-12]

Many episodes of acute diarrhoea in infants become associated with lactose intolerance because of damage to the proximal small intestinal mucosa, where lactase activity is maximal. For this reason, lactose-free formulae are helpful in the management of patients who develop this condition. Many probiotics exhibit lactase activity which remains functional in the gastrointestinal tract and may

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improve the symptoms of intolerance in individuals with hypolactasia.[13,14]

The aims of this study were to determine the effect of a lactose-free formula supplemented with *St. thermophilus* and the probiotic *B. lactis* Bb12 at concentrations of 10^8 or 10^9 CFU per gram of powder on the duration of episodes of acute diarrhoea in infants.

SUBJECTS AND METHODS

Experimental design

The study was carried out at the Department of Paediatrics, the Second University Hospital in Chengdu, China. Infants between 6 and 36 months of age admitted to the Ward because of severe acute diarrhoea and free from moderate or severe malnutrition (weight/age after rehydration at least 75% of the 50^{th} percentile of the NCHS reference charts) were enrolled. They received initially parenteral and/or oral rehydration with oral rehydration solution (ORS) for fluid and electrolyte replenishment according to the rules established by the Department of Paediatrics. The protocol of the project was approved by the Ethics Committee on Research in Humans of the Second University Hospital; China University of Medical Sciences, Chengdu, Sichuan, P.R. of China. The parents of the infants included in this study received careful and complete explanations about the protocol and agreed to the participation of their sons/daughters.

A double-blind, randomised, placebo controlled design was used for the study. The criteria for exclusion were: total or partial breastfeeding, diarrhoea of more than 48 hours duration, the need for antibiotic treatment, severe malnutrition, documented allergy to cow's milk and gastrointestinal or other chronic pathologies that may interfere with the study. After the experimental design had been carefully explained to the parents and their written consent had been obtained, 224 children (mean age: 13.1 ± 0.5 months) were randomly allocated to one of the three experimental groups, usually after 12 to 24 hours of oral and/or parenteral rehydration. These three groups were refed with: 1) a milk-based lactose-free formula (AL 110, control); 2) the same formula but supplemented with viable lyophilised *B. lactis* Bb12 and *St. thermophilus* TH4 (both from Chris Hansen, Denmark) at approximately 10^8 CFU/g and $5x10^7$CFU/g respectively (Bb12-10^8 group) and, 3) the same formula supplemented with the same microorganisms but at approximately 10^9 CFU/g and $5x10^8$, respectively (Bb12-10^9 group). Their administration was concluded 24 hours after the diarrhoea was considered to have ended.

Viability of the microorganisms in the formulae was determined by plate counting. Aliquots of formula were serially diluted in sterile saline, and plated on M17 agar plates (for *St. thermophilus*) and on modified MRS containing 5% glucose, 0.1% LiCl, 0.05% cystein and 0.5 mg/ml dicloxacillin (for Bb12). Plates were incubated under anaerobiosis at 37ºC for 48 hours and CFU were counted.

On enrolment the duration of the episode of diarrhoea and the number of stools per day before admission were ascertained by questioning the parents. Acute diarrhoea was defined as the passage of one watery or mucous stool or three or more loose stools daily or for more than 24 hours. Stool frequency and consistency were recorded daily until day 7. Diarrhoeal duration was defined from the time of admission to the time of the first formed stool if followed by two consecutive non watery stools or by 12 hours without further evacuations. Failure of treatment was defined as continuous or increased severity of diarrhoea with ongoing weight loss or deteriorating fluid and electrolyte balances. A relapse was defined by the reappearance of liquid stools up to 15 days after the original episode had appeared.

Body weight and length, the degree of dehydration, ORS and formula intake or parenteral fluid volumes, the plasma concentrations of sodium, potassium and chloride were measured as well as the status of the acid-base balance. Bodyweight was determined after 8, 12 and 24 hours and then on days 2, 3, 4, 5, 6 and 7 after admission.

The aetiology of the diarrhoea was evaluated by standard culture methods and the presence of rotavirus was detected in fresh stool samples on admission and on day 5, using a semi-quantitative double sandwich ELISA (Lanzhou Institute of Biological Products, Gansu, P. R. of China) according to the manufacturer's instructions. Results of the ELISA were expressed as "0" absent, "+" moderate and "++" intense". To evaluate the effect of treatment on rotavirus shedding over time, the subjects were classified at the beginning and at the end of the intervention period based on the results of the two ELISA tests (at the beginning and at the end of the study). The differences in the reading of the two rotavirus scores were expressed as follows: "0" for no change; "+1" for a change from "+" to "0" or from "++" to "+"; "+2" for a change from "++" to "0"; "-1" for a change from "0" to "+1" or from "+" to "++" and "-2" for a change from "0" to "++". One response was thus obtained for each subject, with five possible levels from "-2" to "+2" reflecting the worsening or the improvement of the rotavirus shedding.

Statistical analysis

Data on anthropometric, clinical, acid-base balance and plasma electrolyte parameters were expressed as means ± SEM. The number of stools was logarithmically transformed and expressed as geometric means and range. Groups were compared using Kruskal-Wallis analysis of variance on ranks or Chi-square test.

To analyse the data on rotavirus shedding, a multinomial model function from the Venables and Ripley Library was used to fit these data and to obtain the probabilities of improvement of virus excretion for each treatment.

RESULTS

A total of 212 infants (71 in the control and the same number in the Bb12-10^8 groups and 70 in the Bb12-10^9 group) were included in the protocol. No dropouts occurred during the study but 12 infants were excluded *a posteriori* because they did not match some of the inclusion criteria (five were less than 3 months of age and seven were older than 36 months). As shown in Table 1, the non parametric analysis of variance (Kruskal Wallis Anova) did not show any differences on enrolment between the groups in relation to anthropometric data, acid-base parameters and plasma levels of sodium, chloride

Table 1. Anthropometric, biochemical and clinical parameters on enrolment in the three groups of children.

	Control (n=71)	Bb12-10^8 (n=71)	Bb12-10^9 (n=70)	*p* (Kruskal Wallis ANOVA)
Age (month)	13.4 ± 0.4	13.2 ± 0.7	12.6 ± 0.7	n.s.
Sex (% male)	52	58	56	n.s.
Weight (g)	9836 ± 194	9877 ± 214	9401 ± 156	n.s.
Height (cm)	74.3 ± 0.8	75.5 ± 0.9	73.7 ± 0.8	n.s.
Weight/Height z score	0.53 ± 0.20	0.21 ± 0.22	0.19 ± 0.21	n.s.
Weight/Age z score	-0.13 ± 0.12	-0.04 ± 0.15	-0.31 ± 0.12	n.s.
Plasma K^+	4.13 ± 0.05	4.13 ± 0.06	4.12 ± 0.04	n.s.
Plasma Na^+	143 ± 0.5	144 ± 0.6	142 ± 0.7	n.s.
Plasma Cl^-	102 ± 0.6	103 ± 0.6	102 ± 0.6	n.s.
Plasma $HCO3^-$	21.5 ± 0.3	21.9 ± 0.2	21.5 ± 0.2	n.s.
pH	7.39 ± 0.004	7.38 ± 0.004	7.39 ± 0.004	n.s.
ORS volume intake (mL)	229 ± 14	206 ± 13	211 ± 13	n.s.
Total Nº of stools/day (Geom. mean [range])	5.6 [2-20]	7.1 [3-20]	6.7 [3-30]	n.s.
Nº of liquid stools/day (Geom. mean [range])	1.5 [1-10]	1.4 [1-5]	1.4 [1-4]	n.s.

(Mean ± SEM). n.s. = not significant (p>0.05); SEM=standard error of the mean

Table 2. Effect of formula intake on some characteristics of diarrhoea.

	Control (n=71)	Bb12-10^8 CFU/g (n=71)	Bb12-10^9 CFU/g (n=70)	*p* (Kruskal Wallis Anova)
Number of stools/day (Geom. Means [range]	3.9 [1.5-12.0]	4.0 [2.0-10.7]	3.9 [1.7-12.6]	n.s.
Duration of diarrhoea (days)	2.8 ± 0.2	2.7 ± 0.1	2.8 ± 0.2	n.s.

n.s.=not significant.

Table3. Effect of treatments on rotavirus shedding expressed as probabilities of improvement (%).

Class	Control (n=71)	Bb12-10^8 (n=71)	Bb12-10^9 (n=70)
-2	0	0	0
-1	0	0	1.5
0	28.6	33.9	15.4
+1	57.1	41.9	69.2
+2	14.3	24.2	13.8

"0" means no change when comparing initial and final results; "+1" represents changes from "+" to "0" or from "++" to "+"; "+2" represents changes from "++" to "0"; "-1" represents changes from "0" to "+1" or from "+" to "++" and "-2" represents a change from "0" to "++".

and potassium. The duration of the diarrhoea (68 ± 4h, 68 ± 4h and 66 ± 3h in the control, Bb12-10^8 and Bb12-10^9 groups, respectively) as well as the total number of stools/day and the number of liquid stools/day were not statistically different (Table 1). Of the total number of diarrhoeal episodes, 13% were associated with the presence of bacterial pathogens and 87% with rotavirus, without significant differences between the groups (data not shown). No differences between the groups were observed in relation with the severity of dehydration on enrolment (data not shown), nor in ORS volume intake (Table 1).

As shown in Table 2, neither the duration of the episodes of diarrhoea or the average number of stools/day over the whole episode were significantly different when comparing the three treatment groups; these results remain unchanged when the groups were subdivided according to the degree of dehydration on admission (data not shown). Compared with the control group, a significant weight gain was observed after 24 hours of treatment in the Bb12-108 group but not in the Bb12-109 group (27.5 ± 4.4 g/kg B.W., 38.3 ± 4.5 g/kg B.W. and 27.8 ± 5.0 g/kg B.W., respectively, p=0.012). This difference, however, disappeared on day 3. Volumes of parenteral fluid, ORS and formula were not significantly different between the three groups during any of the seven days of observation (data not shown). Results of rotavirus shedding are shown in Table 3. A slightly higher probability of improvement (decrease) of rotavirus shedding was observed for the Bb12-109 group.

DISCUSSION

Acute diarrhoea is a major cause of infant morbidity and mortality in developing countries, involving high costs for the families and society. Rotavirus is the most important agent causing this condition in infants in many countries.[15] The development of effective methods to manage acute gastroenteritis remains an important goal related to infant health; probiotics have been proposed as useful tools in this respect.[12] The multiplication of pathogenic microorganisms within the gastrointestinal tract is

dependent on microbial and host factors.[16] One such factor is the composition of the autochthonous intestinal microbiota, of which bifidobacteria are one of the dominant components in infants.[7] It is thought that these bacteria exert some of the protective effects against diarrhoea associated with breastfeeding. In addition, bifidobacteria may decrease viral shedding and delay the clinical onset of rotavirus infection in animal models.[17,18]

We carried out a double-blind, randomized, placebo-controlled trial to evaluate the efficacy of a lactose-free infant formula containing *B. lactis* Bb12 and *St. thermophilus* TH4 for the treatment of acute diarrhoea in infants who were admitted to hospital. The results of this study show that the duration of acute diarrhoea in infants is not influenced by intake of the formula containing Bb12 and TH4 at two different concentrations. The only differences observed referred to a mild decrease of rotavirus shedding associated with the probiotic intake, compared to the control formula. The fact that Bb12 intake at the higher concentration is associated with the decrease of rotavirus shedding is of importance considering that at the time the diarrhoea becomes manifest. At this time the virus has already damaged the enterocytes and its shedding may be decreasing spontaneously: the effect of Bb12 is added to and improves this natural evolution. These results confirm some of the findings by Saavedra et al. who, in a double-blind, randomised, placebo-controlled trial carried out in 55 infants aged 5-24 months in a chronic medical care hospital during 17 months, showed that 10% of infants who received a formula supplemented with Bb12 and *St. thermophilus* shed rotavirus at some time during the study, compared to 39% of the subjects who received the control formula (p=0.025).[9] Although Saavedra described a lower incidence of diarrhoea with the Bb12 supplemented formula compared with the control formula (7% *vs.* 31%, respectively, p=0.035), he did not observe any differences between both groups in the duration of the diarrhoea.[9] In this respect, our patients behaved like those of the Saavedra's study. Another study carried out in Thailand by Phuapradit et al. confirms the protective effect of Bb12 on rotavirus infection through the measurement of specific sIgA in saliva.[19]

In relation with the mechanism explaining these effects, some studies using *Lactobacillus* GG showed that it promotes the recovery from rotavirus diarrhoea via augmentation of the local immune response. The increased specific sIgA response to rotavirus is probably relevant in protection against new infections.[10,11] On the other hand, some probiotic strains, including Bb12, have been shown to inhibit, displace or compete with pathogens in their adhesion to intestinal mucus.[20] This mechanism may be operating in the protective effect observed in this study.

As the three formulations tested were lactose-free, there was no interference from this disaccharide and the effects observed can be attributed to the probiotic.

In conclusion, our study shows that, the addition of Bb12 and TH4 does not influence the duration of diarrhoea in infants, but the higher concentration of both microorganisms are associated with a higher probability of decreased rotavirus shedding. This finding probably is of epidemiological importance in the transmission of this viral agent.

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AUTHOR DISCLOSURES

Meng Mao, Tao Yu, Ying Xiong, Zhiling Wang, Hanmin Liu, Martin Gotteland and Oscar Brunser, no conflicts of interest.

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補充雙歧桿菌和鏈球菌的無乳糖牛奶配方對急性腹瀉的效應

益生菌已被推薦用於嬰兒急性腹瀉的管理與預防上。在 224 位 6-36 個月大有嚴重急性腹瀉且沒有中度或嚴重營養不良的嬰兒上，進行一雙盲、隨機及安慰劑控制組的試驗。在由口或靜脈補充水分後，分為 3 組：無乳糖配方(控制組)；無乳糖配方公克奶粉加上 10^8CFU *B. lactis* Bb12 和 $5x10^7$CFU *St. thermophilus* TH4；無乳糖配方分別加上 10^8CFU *B. lactis* Bb12 與 $5x10^7$CFU *St. thermophilus* TH4。評估體位測量資料、腹瀉持續時間和輪狀病毒量。其中有 87%的事件與輪狀病毒感染有關聯。腹瀉持續時間不受益生菌影響。然而，在餵食添加 10^9 Bb12/g 的配方奶的嬰兒上觀察到輪狀病毒量下降，使用這個物質可能具有流行病學的重要性。

關鍵字：嬰兒、急性腹瀉、輪狀病毒、益生菌、乳酸雙歧桿菌 Bb12。