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Fecal Recovery of a Human *Lactobacillus* Strain (ATCC 53103) during Dietary Therapy of Rotavirus Diarrhea in Infants

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Human *Lactobacillus* strain GG (ATCC 53103) as part of the dietary therapy has been shown to shorten the duration of acute rotavirus diarrhea and to potentiate the intestinal immune response against the virus. We studied the ability of *Lactobacillus* GG to survive passage through the gut during rotavirus diarrhea in 29 infants, age range 5.4 to 27.5 months. After oral rehydration, they randomly received either a *Lactobacillus* GG formula or their normal milk ad libitum. All patients who received *Lactobacillus* GG became colonized with the strain as measured by fecal *Lactobacillus* GG counts. This result suggests that *Lactobacillus* GG may promote the establishment of normal intestinal microflora, even during acute gastroenteritis.

Key words: gastroenteritis; rotavirus; probiotics

INTRODUCTION

Rotaviruses are a major cause of acute infantile gastroenteritis world-wide. Symptoms comprise vomiting, fever and watery diarrhea.

The intestinal microflora is considered an important component of the non-immune defense against both pathogenic and potentially pathogenic bacteria, establishing what is called colonization resistance *(14)*. The ecological balance of intestinal microflora is disrupted during acute infections and antimicrobial therapy *(1, 14)*, offending the mucosal barrier functions.

We have demonstrated a significant reduction in the duration of rotaviral diarrhea by administration of human *Lactobacillus* sp. strain GG (*Lactobacillus* GG) *(5)*. This was connected with potentiation of the intestinal immune defense and, furthermore, the rotavirus specific immune response was promoted *(6)*. It has also been shown that a two-week administration of *Lactobacillus* GG to healthy newborns right after birth increases the concentration of intestinal lactobacilli *(4)*. Later, also the administration of *Lactobacillus* GG was shown to colonize the intestinal tract of preterm babies and the safety of the strain in premature babies was established *(13)*. Further studies have verified the shortening of rotavirus diarrhea also in developing countries *(9, 11)*. To investigate whether this type of dietary therapy influences the restoration of colonization resistance, the ability of *Lactobacillus* GG to survive passage through the gut during an episode of rotavirus diarrhea was assessed.

SUBJECTS AND METHODS

Twenty-nine children were enrolled, mean (SD) age 12.4 (5.4) months, admitted for acute gastroenteritis of less than seven days' duration. The study was approved by the Tampere University Hospital Ethical Committee, and the parents gave their informed consent.

Treatment with oral rehydration was followed by rapid refeeding, consisting of foods that the child had consumed prior to the diarrhea (fermented milk products were excluded). Infants still totally breastfed were excluded, as were those with non-rotavirus diarrhea. The patients received randomly either a *Lactobacillus* GG formula (3×10^9 CFU/100 ml), study group (n = 21), or their normal milk, control group (n = 8), ad libitum.

Immediately after testing for rotavirus (Rotazyme®, Abbott Laboratories), study stool samples were obtained. A second study sample was taken during hospitalization (n = 13) or 3–8 d after discharge (n = 5).

Fecal samples were stored at –20°C until analysis (maximum 7 days). Both the total number of lactobacilli and the number of *Lactobacillus* GG (CFU/g of feces) were determined using methods previously described *(10, 12)*.

The fecal sample was weighed in a sterile tube and serial dilutions prepared (10^{-2}–10^{-8}). From each dilution 0.1 ml was seeded on an MRS agar (Labm, UK) plate. The plates were incubated in 10% CO_2 atmosphere for 72 hr and thereafter the colonies of lactobacilli and those typical (large, white, creamy, convex)

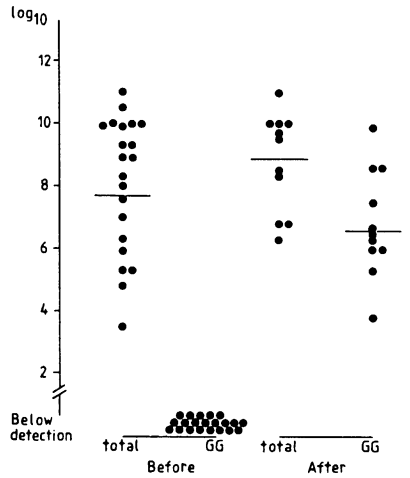

Fig. 1. The $\log_{10}$ number of lactobacilli and *Lactobacillus* GG in the stool of patients before ($n = 21$) and after ($n = 11$) treatment with *Lactobacillus* GG.

of *Lactobacillus* GG were counted. The Gram reaction and cell morphology of *Lactobacillus* GG were determined (Gram positive, uniform rods in chains) and it was further identified as a catalase-negative heterofermentative *Streptobacterium*, able to grow at 15°C but not in 0.4% teepol (Sigma Chemical, St Louis, Missouri, USA) and with negative lactose fermentation reaction.

Because of skewed distributions of fecal bacterial counts, these were transformed to their natural logarithms. In statistical analyses Student's two-tailed *t*-test and Spearman's Rank correlation test were applied.

RESULTS

The mean duration of symptoms at home was 2.8 (1.9) d in the study and 3.0 (1.6) d in the control group, $p = 0.70$. Both groups showed mild to moderate isoosmolal dehydration and metabolic acidosis on admission. Hospital treatment resulted in correction of the dehydration, weight gain, and cessation of the diarrhea in a mean of 1.6 d (range 0.5–4), with no recurrences.

The total number of lactobacilli remained unaltered during the diarrhea and all patients in the study group became colonized with *Lactobacillus* GG (Fig. 1). The amount of *Lactobacillus* GG given did not correlate significantly with that cultured, rho = 0.25, $p = 0.39$.

DISCUSSION

The results show that *Lactobacillus* GG administered perorally during rotavirus diarrhea can be detected in fecal samples in significant numbers. The passage of the strain indicates at least short term colonization during this type of gastroenteritis.

Lactobacilli have been traditionally used in the form of fermented dairy products as a nutritional treatment of a range of clinical conditions, although scientific support for the practice is scanty. Milk fermented with *Lactobacillus bulgaricus* or *Streptococcus thermophilus* is commonly called yoghurt. These lactobacilli do not survive through the intestinal tract *(2, 3)*. *Lactobacillus acidophilus* has been shown to have an impact on the fecal flora of healthy adults, but contradictory results have also been published *(3, 7)*. Such discrepancies could be explained by the difficulties in distinguishing one strain of lactobacilli among the many commonly found in the fecal flora of humans. Thus, each strain should be tested separately for the survival in the gastrointestinal tract and fecal recovery in different clinical situations. Since differences in the duration of diarrhea have been reported even using different strains of the same species, it is important to characterize the factors related to clinical efficacy *(15)*.

In search of ways to prevent and treat enteric viral infections, especially in the immunocompromized patients, new alternatives need to be assessed, such as passive antibodies or protease inhibitors *(8)*. *Lactobacillus* GG has been effective in the treatment of rotavirus diarrhea *(5, 6, 9, 11)* and it has been shown to colonize the feces of healthy and premature newborns. This study shows that the strain can be detected in feces even during rotavirus diarrhea. Our results indicate that colonization of the intestine by *Lactobacillus* GG during acute diarrhea may be connected with the influence on gut local immune response. *Lactobacillus* GG seems a promising candidate for therapeutic stimulation of this defense. Also other strains should be tested for their fecal recovery and clinical effects.

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