

Oral Bacteriotherapy for Viral Gastroenteritis

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The effect of orally administered lactobacilli on acute rotavirus diarrhea was tested in 42 well-nourished children ages 5-28 months. After oral rehydration, the patients were randomized to a study group, receiving human *Lactobacillus casei* strain GG 10^{10} colony-forming units twice daily for five days, or a control group not given lactobacilli. *Lactobacillus* GG was found in the feces in 83% of the study group. The diarrheal phase was shortened in that group. Dietary supplementation with lactobacilli significantly influenced the bacterial enzyme profile: urease activity during diarrhea transiently increased in the control group but not in the study group; $F = 8.6$, $P = 0.01$. No intergroup differences were found in β -glucuronidase, β -glucosidase, and glycocholic acid hydrolase levels. We suggest that rotavirus infection gives rise to biphasic diarrhea, the first phase being an osmotic diarrhea and the second associated with overgrowth of specifically urease-producing bacteria. Oral bacteriotherapy appears a promising means to counteract the disturbed microbial balance.

KEY WORDS: infantile diarrhea; rotavirus infections; *Lactobacillus casei*; clinical.

Viral infections of the gastrointestinal tract in infants and children are a major health problem worldwide. Oral rehydration therapy to correct and maintain the fluid and electrolyte balance during the acute diarrheal episode has substantially reduced the acute complications of gastroenteritis, but with little effect on the course of the acute diarrhea (1). New strategies for the management of acute gastroenteritis are therefore urgently needed. To improve management of rotavirus infection, the leading cause of infantile diarrhea, characterization of sub-

stances that could shorten the period of diarrhea, nutritionally benefit the patient, and strengthen the gut mucosal barrier would be an important breakthrough.

An important constituent of the gut defense barrier is its microflora (2-4). The intestine's mucosal barrier function and microecology are disturbed in acute gastroenteritis (5-8). Changes in gut microecology can be assessed by measuring bacterial enzyme activities in feces (9). Culture-based methods are biased and insensitive; hence a significant proportion of human commensal microflora may be ignored (10). Moreover, the changes in the metabolic activity of the intestinal microflora can occur without changes in the actual numbers or types of microorganisms in the gut. Fecal bacterial enzymes can be used as indicators of changes in intestinal microecology (9, 11, 12). Fecal β -glucuronidase and β -glucosidase are enzymes with a potency to release toxic compounds from substances present in the gut, such as hepatic detoxification products and

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plant glycosides. Such compounds may degrade the mucus layer of the gut, thereby damaging its barrier function (11). Glycocholic acid hydrolase marks bacterial changes related to intestinal inflammation (13). Urease catalyzes the hydrolysis of urea to yield ammonia and carbonic acid. Urease has been considered a proinflammatory agent, and the production of high concentrations of ammonia predisposes to mucosal damage (14, 15).

In the present investigation we administered lactobacilli, which constitute a major part of the microflora throughout the gastrointestinal tract, orally to patients with rotavirus gastroenteritis to reinforce the gut barrier and evaluated the results with regard to the intestinal microecology. We chose *Lactobacillus casei* strain GG (*Lactobacillus* GG), which is of human origin and can survive in the gastrointestinal milieu (16), an important prerequisite for an oral bacteriotherapeutic agent (3). *Lactobacillus* GG has a good safety record from a number of clinical studies, where it has been administered to preterm infants, children, patients with rotavirus diarrhea, adults, and the elderly (16–20).

MATERIALS AND METHODS

Patients and Management. Eligible for the study were children up to 3 years of age with acute diarrhea for less than seven days, more than three watery stools during the previous 24 hr, and rotavirus demonstrated as the cause of the acute gastroenteritis. Informed consent was obtained from the parents and the study was approved by Tampere University Hospital's Committee on Ethical Practice.

On admission the patients were weighed and physically examined. Serum levels of sodium and potassium and blood acid–base balance were determined. Tests for rotavirus antigen in feces were made with an enzyme immunoassay (Rotazyme, Abbott). Feces were cultured for *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia*. Reducing substances were studied in the ward from fresh diarrheal stools using Clinitest (Ames, UK) tablets.

Oral rehydration for 6 hr was followed by rapid reintroduction of full feeding appropriate for the age, including milk and milk products (21), but not in fermented form. The patients were weighed daily and their stools were recorded as watery, loose, or solid. They were discharged according to the pediatrician's judgement and seen again during convalescence.

Study Design. The patients were randomly allocated to two groups, 21 in each. One group received *Lactobacillus* GG as a freeze-dried powder in a dose of 10^{10} colony-forming units (CFU) twice daily for five days. The other, control, group was not given lactobacilli.

Bacterial enzyme activities in feces were assessed at three different stages. Within 6 hr of admission samples were obtained from 16 patients in the study group (before

start of oral bacteriotherapy) and from 12 in the control group. Second samples were taken during oral bacteriotherapy, 30–36 hr after admission, and were obtained from 18 of the study group and 16 of the controls. During convalescence, 21–24 days after admission, eight stool samples were obtained from the study group and six from the control group. These same samples were also used to determine the counts of *Lactobacillus* GG in feces.

Determination of *Lactobacillus* GG in Feces. Total counts of *Lactobacillus* GG in feces were made as described by Saxelin et al (17). Serial dilutions of each sample were incubated anaerobically on MRS agar plates for 78 hr, and the typical large, white creamy colonies were counted. They were finally identified by negative lactose fermentation and typical colony morphology.

Intestinal Bacterial Enzyme Activity. Fecal samples stored at -20°C were thawed at 4°C . About 0.5 g of the sample was transferred into precooled tubes containing 0.1 M potassium buffer (pH 7.0). The samples were homogenized, filtered, sonicated (4×15 sec), and centrifuged at $500 \times g$ for 10 min at 4°C . The supernatant fraction was used for determination of enzyme activities.

Fecal β -glucuronidase (EC 3.2.1.31) and β -glucosidase (EC 3.2.1.21) activities were measured with the Freeman (22) method. The respective substrates were 1 mmol phenolphthalein- β -glucuronic acid (Sigma Chemical Co.) and 2 mmol *p*-nitrophenyl- β -D-glucopyranoside (Sigma).

In the assay of fecal urease (EC 3.5.1.5), the reaction mixture (1 ml) contained 0.02 M potassium phosphate buffer (pH 7.4), 10 mM urea, and 0.2 ml of the fecal supernatant. The assay was run at 37°C and stopped at 10 and 15 min by adding 9 ml of 0.2 N sulfuric acid. Ammonia was determined with a specific ammonia electrode (Orion model No 95-12, Finland) after adding 1 ml of 10 N NaOH.

Glycocholic acid hydrolase (choloal-glycine hydrolase, EC 3.5.1.24) activity was determined with the method of Nair et al (23). The enzyme reaction was run at 37°C in a total volume of 1 ml, containing 0.02 M potassium phosphate buffer (pH 5.8), 2 mM glycocholic acid (Sigma), and 0.1 ml fecal supernatant. It was terminated by adding 1 ml of 15% trichloroacetic acid. The mixture was centrifuged and the supernatant was assayed for glycine with the aid of ninhydrin. Protein was measured in fecal supernatants with the method of Lowry et al (24) and using bovine serum albumin as standard.

Statistical Analysis. Student's *t* test was used for intergroup differences. All hypothesis testing was two-tailed. Results of successive measurements were compared with analysis of variance (ANOVA). Differences in proportions were evaluated with the χ^2 test. Because of skewed distribution, natural logarithmic (ln) transformations were used (25) and data are reported as geometric means with 95% confidence intervals (CI).

RESULTS

Clinical. Forty-two well-nourished patients ages 5–28 months were enrolled in the trial. The mean (SD) age was 13.6 (4.4) months in the study group and 14.4 (5.1) months in the control group. The

LACTOBACILLI IN ROTAVIRUS DIARRHEA

TABLE 1. CLINICAL CHARACTERISTICS AT ADMISSION FOR ACUTE ROTAVIRUS ENTERITIS: RAPID AGE-APPROPRIATE REALIMENTATION AND *Lactobacillus* GG (STUDY GROUP) OR NO LACTOBACILLI (CONTROL GROUP)*

	Study group (N = 21)		Control group (N = 21)		Student's t-test P
Diarrhea at home (days)	3.0	(1.5)	3.2	(1.3)	0.62
Acute weight loss (g)	490	(200)	410	(190)	0.20
Dehydration (%)	4.9	(2.3)	4.7	(2.1)	0.80
Rectal temperature (°C)	38.3	(0.7)	38.3	(0.8)	0.91
Serum					
Na ⁺ (mmol/liter)	138	(4)	139	(4)	0.27
K ⁺ (mmol/liter)	3.9	(0.5)	4.0	(0.5)	0.54
Blood					
pH	7.33	(0.06)	7.33	(0.07)	0.82
Base excess (mmol/liter)	-8.2	(3.3)	-8.1	(4.9)	0.94

*Figures denote means (SD).

groups were comparable as regards clinical history (Table 1). On admission the patients had mild to moderate isoosmolar dehydration with metabolic acidosis (Table 1). Clinitest was positive in half of the stool samples in both the study group and the control group. All cases were successfully managed with scheduled oral rehydration and age-appropriate realimentation (Table 2).

The introduction of *Lactobacillus* GG to the rapid refeeding schedule resulted in reduced duration of diarrhea, which became apparent after the first day of treatment (Table 2). Recovery was uneventful in all cases.

Recovery of *Lactobacillus* GG in Feces. The tests 30–36 hr after admission were positive (7.5×10^3 to 5×10^8 CFU/g) in 15 of the 18 tested patients in the study group. The samples taken before the start of oral bacteriotherapy or during convalescence were negative for *Lactobacillus* GG (detection limit 10^3 CFU). The strain was not detectable in any samples from the control patients.

Intestinal Bacterial Enzyme Activities. The activities of β -glucuronidase, β -glucosidase, and glyco-

cholic acid hydrolase were in general very low (Table 3). The differences in activity levels between the study and the control group were not statistically significant (Student's *t* test). In the diarrheal period there was no significant change in the activities of these enzymes at successive measurements (Table 3). The activities of β -glucuronidase, β -glucosidase, and glycocholic acid hydrolase during convalescence were indistinguishable between study and control groups. At convalescence the β -glucuronidase and β -glucosidase levels were increased in both groups: 1.44 (95% CI 0.62–3.36) nmol/min/mg and 3.21 (1.98–5.21) nmol/min/mg, $P = 0.04$ and $P = 0.08$, respectively. The activity of glycocholic acid hydrolase remained low in both the study and the control group alike: 0.04 (0.005–0.52) nmol/min/mg.

During the diarrheal phase of rotavirus infection,

TABLE 3. FECAL ENZYME ACTIVITIES: MEAN (95% CONFIDENCE INTERVAL) DURING ROTAVIRUS DIARRHEA IN STUDY GROUP (GIVEN *Lactobacillus* GG) AND CONTROL GROUP (NO LACTOBACILLI)*

	Study group (nmol/min/mg)		Control group (nmol/min/mg)	
β -Glucuronidase				
Sample 1	0.01	(0.001–0.07)	0.01	(0.001–0.04)
Sample 2	0.02	(0.004–0.12)	0.06	(0.01–0.33)
β -Glucosidase				
Sample 1	0.02	(0.002–2.68)	0.01	(0.001–0.16)
Sample 2	0.12	(0.001–0.92)	0.66	(0.12–3.80)
Glycocholic acid hydrolase				
Sample 1	0.08	(0.01–0.94)	0.06	(0.002–2.68)
Sample 2	0.003	(0.001–0.01)	0.02	(0.002–0.18)

*Sample 1 ($N = 16$ from study group and $N = 8$ –12 from control group) taken before start of *Lactobacillus* GG treatment. Sample 2 ($N = 15$ –17 from study group and $N = 13$ –16 controls) taken 30–36 hr after admission, during rapid refeeding with or without *Lactobacillus* GG.

TABLE 2. OUTCOME OF THERAPY IN STUDY GROUP (GIVEN *Lactobacillus* GG) AND CONTROL GROUP (NO LACTOBACILLI)

	Study group (N = 21)	Control group (N = 21)	P
Weight gain [g, mean (SD)]	240 (180)	235 (200)	0.99*
Duration of diarrhea, [days, mean (SD)]	1.5 (0.7)	2.3 (0.8)	0.002*
Diarrheal stools in % of patients			
Day 1	100	100	
Day 2	62	86	0.07†
Day 3	10	43	0.01†

*Student's *t* test.

† χ^2 test.

A On admission

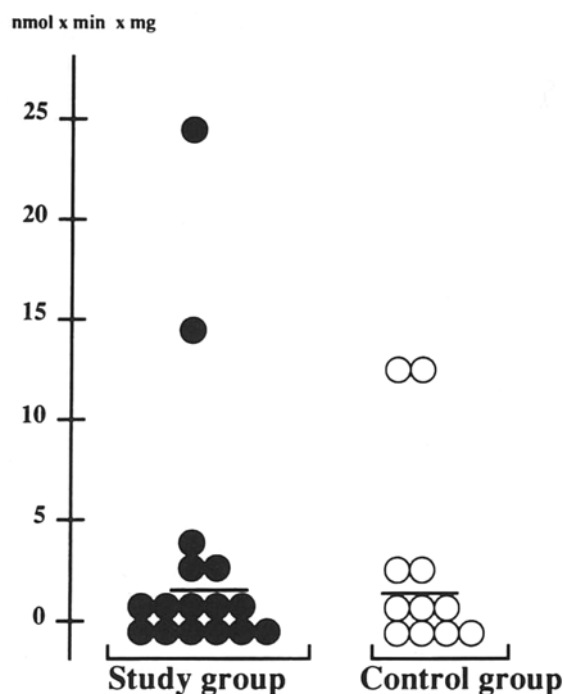


Fig 1. Fecal urease during rotavirus diarrhea in patients given *Lactobacillus* GG freeze-dried powder (study group) and in controls. On admission: 16 samples from study group and 11 from control group were taken before start of *Lactobacillus* GG treatment; 30–36 hours after admission: 17 samples were taken from study group and 16 from control group during rapid refeeding with or without *Lactobacillus* GG.

urease levels (Figure 1) were unaltered in patients who received *Lactobacillus* GG, but increased significantly in the control group. ANOVA for repeated measurements showed significant interaction ($F = 8.60$, $P = 0.01$) between groups and periods, indicating that at these successive measuring points the urease activity levels differed between the study group and the control group. The intergroup difference was manifest even when comparing only the patients with watery stools at the time of the second assessment of urease activity: 0.002 (0.001–0.02) nmol/min/mg in the diarrheal patients receiving *Lactobacillus* GG vs 6.27 (1.2–33.8) in the controls (samples available from 10/13 and 13/18 patients in the respective groups), $t = 6.63$, $P = 0.0001$. The rise in fecal urease activity was transient. In convalescence the level was low, 0.51

B 30 - 36 hours after admission

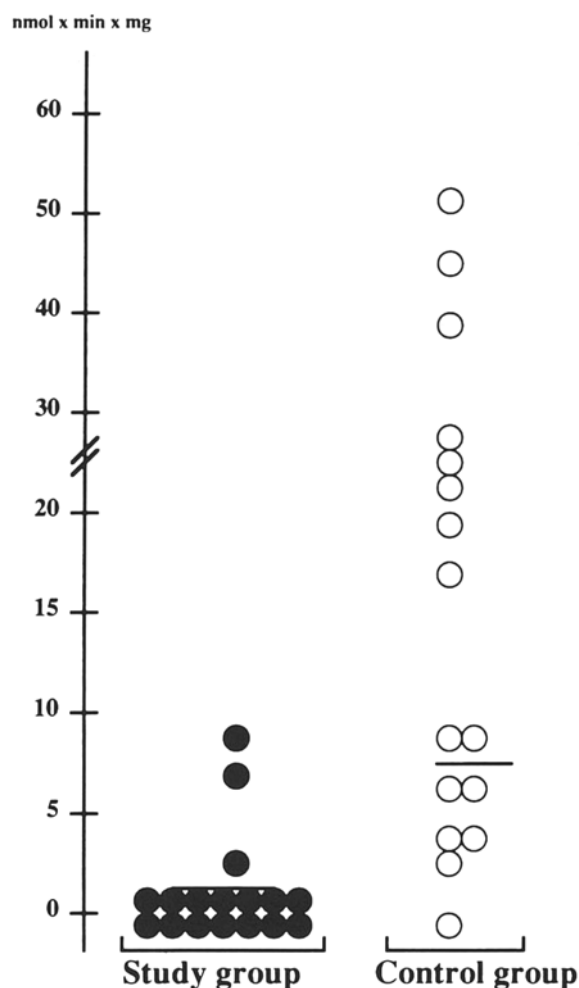


Fig 1. Continued.

(0.03–8.30) nmol/min/mg, and there was no difference in urease activity between the study group and the control group.

DISCUSSION

The results of the present study provide indirect evidence of disturbed intestinal microecology in children with rotavirus gastroenteritis. They further suggest that intestinal microfloral balance is connected with early cessation of rotavirus diarrhea. We observed that urease activity increased transiently after a period of noticeably low bacterial enzyme activity, while the levels of β -glucuronidase, β -glucosidase, and glycocholic acid hydrolase remained unaltered. These observations suggest

susceptibility to overgrowth of specifically urease-producing bacteria in rotavirus diarrhea.

In rotavirus gastroenteritis, infection of mature, differentiated enterocytes lining the villi of the small intestine leads to patchy mucosal lesions (26). There is shortening of the villi with consequent reduction of villous enzymes, such as sucrase and lactase, and increase in crypt depth. The infected enterocytes are replaced by immature crypt-type cells with reduced absorptive function. Diarrhea induced by rotavirus closely resembles that seen in malabsorption syndromes. Osmotic diarrhea results from malabsorption of carbohydrate, and acidity of the stools is due to organic acids generated in the large bowel by bacterial fermentation of malabsorbed carbohydrates (27, 28).

In contrast to rotavirus diarrhea, bacterial diarrhea seems to be associated with alkaline stools (6, 29). The rise in pH of the intestinal contents may be due to hydrolysis of urea by bacterial urease, yielding ammonia and carbonic acid, which will enhance the survival of acid-sensitive organisms (30, 31) and reduce the numbers of anaerobes (29). Reduction of anaerobes increases the vulnerability of the host to infections, and ammonia in high concentrations can also predispose to mucosal damage (15). The results of the present study suggest a more indirect interference in rotavirus diarrhea. The disturbance of the intestinal microecology, as indicated by increased urease activity, and the features of rotavirus diarrhea may be reconciled as follows. The primary event, the infection by rotavirus, rapidly causes osmotic diarrhea with acidic stools. The acidity of the colonic contents converts ammonia to ammonium ion, thus preventing its absorption. A study of rat colonic tissue (32) showed that NH_3 is approximately 400 times more permeating than NH_4 . Finally, unabsorbed ammonium ion will provide nitrogen to many enteric bacteria (14), among others to urease-producing bacteria. Therefore, the factor predisposing the host tissues to the bacterial overgrowth may be the loss of protective anaerobic microflora following rotavirus-induced intestinal dysfunction. We therefore suggest that rotavirus infection gives rise to biphasic diarrhea, the first phase being an osmotic diarrhea and the second associated with bacterial overgrowth.

The hypothesis that rotavirus infection is followed by bacterial overgrowth resulting in a second phase of diarrhea is supported by a prospective comparison between breast-fed infants and others receiving adapted cow milk formula during a rota-

virus epidemic (33). The rate of rotavirus infection did not differ between the groups. The clinical disease was milder in the breast-fed infants, in 20.5% of whom significant growth of bifidobacteria was maintained during rotavirus diarrhea, while such colonization was not found in the formula-fed infants. In healthy adults *Bifidobacterium longum* was shown to reduce the pH and the ammonia concentration in feces (34).

Research on rotavirus gastroenteritis in infants is currently focused on optimal diet (35). In the present study the refeeding diet strongly influenced the bacterial enzyme profile: dietary supplementation with lactobacilli counteracted the rise in urease activity and shortened the diarrheal phase, the latter finding confirming our previous clinical observations (18, 19). The decrease in urease activity was not due simply to earlier cessation of diarrhea, as urease activity was lower in the study patients who still had diarrhea than in the control group. Moreover, it is unlikely that the refeeding diet *per se* enhanced urease activity in control patients.

Collectively these results indicate that the microflora is important in the intestinal defense system. Lactobacilli can be regarded as a safe and promising candidate for oral bacteriotherapy. Our observation that oral bacteriotherapy counteracted disturbance of microbial balance points to the need for further studies in malnourished infants at risk of sequelae from viral gastroenteritis.

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