Lactobacillus reuteri as a Therapeutic Agent in Acute Diarrhea in Young Children

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Abstract

Background: Certain strains of lactobacilli may promote recovery from acute diarrhea. Lactobacillus reuteri is of human origin and is a natural colonizer of the gastrointestinal tract. In this trial, exogenously administered L. reuteri was studied as a therapeutic agent in acute diarrhea.

Methods: Forty patients between 6 and 36 months of age hospitalized with acute diarrhea (75% rotavirus) were studied. After parental consent, the patients were randomized to one of two treatment groups to receive either $10^{10}$ to $10^{11}$ colony-forming units of L. reuteri or a matching placebo daily for the length of hospitalization or up to 5 days. The clinical outcome of diarrhea and colonization of the gastrointestinal tract were evaluated.

Results: The mean (SD) duration of watery diarrhea after treatment was 1.7 (1.6) days in the L. reuteri group and 2.9 (2.3) days in the placebo group (p = 0.07). On the second day of treatment only 26% of patients receiving L. reuteri had watery diarrhea, compared with 81% of those receiving placebo (p = 0.0005). Cultures of lactobacilli from stool samples demonstrated that administration of L. reuteri resulted in colonization of the gastrointestinal tract. Lactobacillus reuteri accounted for >75% of the total lactobacilli found in children fed with this product.

Conclusions: Lactobacillus reuteri is effective as a therapeutic agent in acute rotavirus diarrhea in children. Further studies are warranted to confirm the present finding and to explore the full therapeutic potential of L. reuteri in acute viral diarrhea.

Normal microflora is important in the protection of the host against diseases of the gastrointestinal tract (1-3). During periods of acute diarrhea, the normal gastrointestinal microflora is radically changed, including decrease of Lactobacillus, Bacteroides, and Bifidobacterium species (2-5). Several studies have indicated that the administration of probiotic agents may modulate the microbial balance of the host and attenuate acute episodes of diarrhea (6-8). Lactobacillus strain GG (LGG) has been shown to promote clinical recovery from rotavirus gastroenteritis in children and enhance intestinal immune responses (9-11). Other commercially available preparations of lactic acid bacteria, such as L. casei subsp. rhamnosus (Lactophillus), L. delbrückii subsp. bulgaricus, and others are also being used for the treatment of acute diarrhea, even though their efficacy has not been formally demonstrated (11).

Lactobacillus reuteri was primarily isolated from human breast milk, and is the most commonly occurring Lactobacillus species found in the gastrointestinal tracts of humans and animals (12). Like other lactobacilli, L. reuteri produces acidic metabolic endproducts (lactic and acetic acids) that have considerable antimicrobial activity (13). L. reuteri is known to colonize the human intestine after consumption of milk products; such products are actually being marketed in Sweden. Therefore, L. reuteri can be produced in an easily consumable form for use in the management of diarrhea, if it is proved efficacious.

L. reuteri has been shown to be safe on exogenous administration to healthy human adults (14) and hospitalized children (A. Shornikova et al.,...
unpublished) and has shown therapeutic potential in a rat model of colitis (15). In this study we have assessed L. reuteri as a therapeutic agent in acute diarrhea of young children, largely associated with rotavirus, and studied the extent of total lactobacilli and L. reuteri colonization of the gastrointestinal tract with and without exogenous L. reuteri. In addition, we have investigated the effect of L. reuteri on the immune response to rotavirus after diarrhea associated with rotavirus.

MATERIALS AND METHODS

Patients and Study Design

The study was carried out between January 29 and July 3, 1995, corresponding to a rotavirus epidemic season. This was a randomized, double-blind study. Study subjects included 41 well-nourished patients (61% male) between 6 and 36 months of age consecutively admitted to the Department of Pediatrics, Tampere University Hospital for acute diarrhea of <7 days' duration who had had one or more watery stools during the preceding 24 h. Children were enrolled in or excluded from the study on the basis of the following inclusion and exclusion criteria. Patients were eligible for study if they were 6-36 months of age, were admitted for acute diarrhea, had a history of ingesting bovine dairy products (milk, yogurt, infant formula, etc.) as part of their normal diet, and had a parent or legal guardian who signed an informed consent. Patients were excluded from study if they were taking immunosuppressive therapy or suffering from immune deficiency, had a history of allergy to bovine milk, had a serious underlying disease, had taken an investigational product during the preceding month, or had a parent or legal guardian who refused to sign an informed consent.

A randomization schedule was prepared to assign approximately 50% of enrolled patients to each treatment group (L. reuteri and placebo). Randomization numbers were sequentially assigned to patients as they were enrolled in the study.

At the time of admission, the children were weighed and clinically examined, and the severity of dehydration was estimated. Acute weight loss was calculated as the difference between expected weight (according to individual growth charts) and observed weight. Fluid deficit (dehydration percent) was then defined from the clinical signs of dehydration and acute weight loss with a reduction of 0.5 to 1% per day if diarrhea had continued for at least 3 days to reflect loss of weight due to low caloric intake. Serum levels of sodium and potassium as well as the blood acidbase balance were determined from a blood specimen collected on admission.

After admission to hospital, the patients were managed according to a standard treatment practice, first with oral rehydration followed by rapid resumption of full feeding (16), but without anti diarrheal drugs. Oral rehydration was accomplished in 6 h with a solution containing Na+ 60 mmol/L and glucose 84 mmol/L (Light ORS), given at two times the fluid deficit, with a minimum of 30 ml/kg (17). After initial rehydration, ORS was administered to replace ongoing losses in diarrhea or vomiting.

Patients were equally randomized to one of two groups. Group 1 (n = 19) received 10^10-11 CFU of L. reuteri SD 2112 once a day. Group 2 (n = 21) received a matching placebo once a day. The placebo consisted of nonfat dry milk powder. L. reuteri and placebo formulations were prepared, quality controlled, and quality assured by Bio-Gaia Biologics, Inc. (Raleigh, NC, U.S.A.) prior to shipping. Each 1-g dose containing 10^10-11 CFU per gram of L. reuteri was packaged in freeze-dried form in sterile sealed plastic vials using nonfat dry milk powder as a carrier. During the study the preparations were stored at -20°C. One-gram freeze-dried preparations of L. reuteri or placebo were reconstituted in 50-100 ml of a fluid of choice. Hot food was tempered before mixing with the formulations. The feeding of the assigned preparation was started immediately after the informed consent had been obtained. The patients received L. reuteri or placebo for 5 days or for the duration of hospitalization, if shorter.

The patients were weighed after rehydration and daily thereafter in the ward. The number and quality of the stools and the number of vomiting episodes were recorded by attending nurses. The stools were recorded as watery, loose, or solid. The duration of diarrhea was counted up to the last appearance of watery stools and measured as decimal days. The patients were discharged according to the clinical judgment of the attending physician. They were asked to contact the investigators if diarrhea recurred...
in a follow-up period of 1 month, at which point they were seen again for the collection of a blood specimen.

**Laboratory Methods**

Concentrations of serum sodium, potassium, and blood acid-base analysis were determined in the hospital laboratory using standard procedures.

Rotavirus antigen was demonstrated using a commercial enzyme immunoassay (Dakopatts AS, Denmark) in the Department of Virology, Medical School, University of Tampere. Blood specimens for rotavirus serology were collected on the same day or one day after admission and four weeks later. Rotavirus IgA class antibodies were determined using an ELISA method [18].

Stools were collected from each subject for analysis of total lactobacilli and *L. reuteri*. Fecal samples were collected at baseline prior to study product administration, 48 h after study product administration, and at hospital discharge. No less than 2 g of stools were collected for microbial analysis. The samples were homogenized and diluted in 0.1% peptone water for a final ratio of 1:5. Five aliquots of 1.6 ml each of well-mixed preparations were quick-frozen and stored at -70°C. Diluted stool samples were sent to BioGaia Biologics, Inc., Raleigh, NC, U.S.A. for determination of the total fecal lactobacilli and *L. reuteri*.

The urease activities in feces were determined in the laboratory of Clinical Nutrition Department, University of Kuopio, as previously described [19,20].

**Statistical Methods**

Statistical analysis was performed using StatView IV program. Student’s two-tailed *t* test, analysis of variance, and chi-square test were used in the determination of statistical differences between study groups. A paired *t* test was used to compare the results of repeated measurements. Because of skewed distribution of the fecal urease activities, the data presented are the median of measurements within the interquartile range (IQR) from the 25th to the 75th percentile. The nonparametric Wilcoxon test was applied in this analysis.

**Ethical Considerations**

The study protocol had been approved by the Ethical Review Committee of Tampere University Hospital.

**RESULTS**

A total of 41 patients were initially enrolled in the study. One child in the placebo group was removed from the analysis because rotavirus was found in the stool samples. His sister, also included in the trial, was assigned to the group. It was obvious that cross-contamination had taken place between these children. Of the remaining 40 children, 19 and 21 patients were assigned to the *L. reuteri* and placebo treatments, respectively. Thirty (75%) patients had rotavirus antigen in the stool specimens by enzyme immunoassay. Rotavirus was found in the *L. reuteri* group from 12 (63%) and in the placebo group from 18 (86%) patients.

In the total study group (n = 40) the mean (SD) duration of diarrhea until treatment was 3.0 (1.7) days. On admission most patients had mild dehydration, mean 3.4 (1.4)% The serum sodium was between 130 and 144 mmol/L, with a mean of 138 mmol/L. The rotavirus-positive patients (n = 30) had had diarrhea for 2.6 (1.5) days at home, compared with 3.1 (1.9) days in the 10 rotavirus-negative patients (difference not significant). The degree of dehydration in rotavirus-positive children was not significantly more severe than in rotavirus-negative patients, but they had more metabolic acidosis (base deficit 7.8 (4.3) mmol/L) than the non-rotavirus patients on admission (mean base deficit 4.8 (3.8) mmol/L), respectively (*p* = 0.07). The characteristics of patients receiving *L. reuteri* or placebo are presented in Table 1. On admission, the groups were comparable except that the patients in the *L. reuteri* group were more dehydrated than those in the placebo group.

**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo group</th>
<th>L. reuteri group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydration (mean)</td>
<td>3.4%</td>
<td>3.1%</td>
</tr>
<tr>
<td>Serum sodium (mean)</td>
<td>138 mmol/L</td>
<td>138 mmol/L</td>
</tr>
<tr>
<td>Rotavirus-positive patients</td>
<td>18 (86%)</td>
<td>12 (63%)</td>
</tr>
</tbody>
</table>
The clinical outcome of the two treatments were similar for weight gain, correction of acidosis, and electrolyte levels (Table 2). The mean duration of watery diarrhea was shorter in the *L. reuteri* group (p = 0.07). Days 0, 1, 2, 3, 4, 5, and 6 were calculated as 24-h periods before or after the initiation of *L. reuteri* or placebo treatment. The effect of *L. reuteri* on persistence of watery diarrhea is further presented in Table 3 and Fig. 1. By the second day of treatment, watery diarrhea persisted in only 26% of *L. reuteri* recipients compared with 81% of placebo recipients. On day 2 the mean frequency of watery diarrhea was 1.0 (SD 2.3) in the *L. reuteri* group and 2.5 (SD 2.3) in the placebo group (p = 0.05) (Fig. 1), and on day 3 the mean frequencies were 0.5 (SD 1.9) and 1.7 (SD 2.6) in the *L. reuteri* and placebo groups, respectively (p = 0.12).

Fewer patients receiving *L. reuteri*, compared with those receiving placebo, had vomiting, starting from the second day of treatment (Table 4). Vomiting practically stopped after the first day of therapy in the *L. reuteri* group, while in the placebo group it continued in a few patients until day 6.

The administration of *L. reuteri* resulted in good colonization of the gastrointestinal tract. A net increment of 5 log of *L. reuteri* in feces was observed after 48 h of *L. reuteri* administration. Total lactic acid bacteria also showed a 2 log increase after 48 h (Table 5). *L. reuteri* accounted for more than 75% of total lactic acid bacteria detected in stool samples. Total lactobacilli were low in the stools of placebo-treated children, and *L. reuteri* was not detected in any of these stool samples. Throughout the study total fecal...
lactobacilli from placebo-treated children were in the range of 10^-10 CFU/g.

<table>
<thead>
<tr>
<th>Time</th>
<th>Fecal lactobacilli (log CFU/g)</th>
<th>L. reuteri L. reuteri</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>2.2 (1.6-3.0)</td>
<td>1.9 (1.6-2.0)</td>
</tr>
<tr>
<td>Disch.</td>
<td>6.0 (5.0-7.0)</td>
<td>6.0 (5.0-7.0)</td>
</tr>
</tbody>
</table>

The urease activities in feces were very low during the peak of rotavirus diarrhea. The median urease levels increased in patients receiving the placebo (n = 9) from 1.23 (IQR 0-23.66) nmol/min/mg on admission to 4.25 (IQR 0-7.72) on day 2, but there was no such increase in those receiving L. reuteri. In the L. reuteri group (n = 12), the median urease activity on admission was 0 (IQR 0-2.09) nmol/min/mg, and on day 2 it was 0.96 (IQR 0-6.05) nmol/min/mg.

Rotavirus IgA antibodies were similar in the two study groups. On admission the mean rotavirus IgA antibody level in the L. reuteri group was 22.6 (SD 39.8) enzyme immunounits (EIU), and in the placebo group it was 7.99 (SD 21.8) EIU (p = 0.16). Four weeks later the mean rotavirus IgA antibody levels were 74.2 (SD 33.9) and 66.3 (SD 31.9) (p = 0.47) in the L. reuteri and the placebo groups, respectively.

**DISCUSSION**

The clinical results, substantiated by fecal analysis, indicate that the colonization of L. reuteri in the GI tract resulted in shortening and amelioration of acute diarrhea, mainly of rotavirus etiology. The benefits of L. reuteri therapy were observed within 24 h of the onset of treatment, after which a reduction of watery diarrhea was seen in most patients. The observation that 74% of the treated patients and only 19% of placebo patients were free of diarrhea on the second day of therapy is clearly of clinical significance. The present study was of limited size and requires confirmation. The results might actually be further improved in a trial designed for earlier administration of L. reuteri. In the present study, L. reuteri therapy was started at a relatively late stage of diarrhea when the patients required hospitalization, and even so only after completion of rehydration and securing the parental consent.

The clinical results were corroborated by the bacteriological findings, which indicated a low total number of lactobacilli and virtual absence of L. reuteri in the placebo recipients, and high total lactobacilli and colonization of L. reuteri in the treatment group. The colonization data suggests that the presence of L. reuteri in the gastrointestinal tract may improve gut ecology by facilitating the growth of other beneficial microorganisms (1). Altogether, L. reuteri may be regarded as an effective colonizer, in line with Lactobacillus GG (21), and it may survive better than most lactic acid bacteria in the gastrointestinal tract (21,22).

Enzyme activities in feces may correlate with changes in intestinal microflora during diarrhea (19). Our results confirm a previous finding (19,20) that rotavirus infection is followed by overgrowth of urease-producing bacteria, which may contribute to prolongation of diarrhea. Indirect evidence of suppression of such bacterial growth was obtained by the demonstration of low fecal urease levels in L. reuteri-treated patients. L. reuteri is known to produce a broad-spectrum antimicrobial, called reuterin (7), which may be responsible for the inhibition of pathogenic microorganisms in the gastrointestinal tract.

In addition, the beneficial clinical effect of L. reuteri may result from other mechanisms, such as stabilization of the mucosal barrier with a decrease in intestinal permeability (23,24) and stimulation of intestinal immune responses (12,25,26).

This study failed to find evidence of an immunostimulatory effect of L. reuteri, as convalescent-stage rotavirus-specific serum IgA antibody responses were similar in L. reuteri and placebo recipients. However, this observation does not exclude the possibility of stimulation of immune response.
mechanisms by \textit{L. reuteri}, since other modalities of host immune response were not investigated.

In conclusion, \textit{L. reuteri} appears a promising therapeutic agent for treatment of acute gastroenteritis, particularly rotavirus-associated disease, in young children. The therapeutic potential should be explored further for possible practical clinical applications. In addition, \textit{L. reuteri} may have other beneficial health effects, including prevention of diarrheal disease. Investigations of the latter are currently under way.

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**Annual Topics in Gastroenterology and Liver Disease**

September 24-26, 1997, Baltimore, Maryland, U.S.A., sponsored by The Johns Hopkins Medical Institutions and Meyerhoff Center for Digestive Disease. This annual postgraduate seminar for gastroenterologists, endoscopists, internists, and surgeons interested in the treatment of digestive and liver disease will focus on peptic ulcer therapy, helicobacter pylori, methotrexate use in IBD, other IBD therapies and surgery, laparoscopic cholecystectomy, hepatobiliary, pancreatic surgery, liver transplantation, hepatitis C, and other chronic liver diseases. The Johns Hopkins University School of Medicine is accredited by the Accreditation Council for Continuing Medical Education to sponsor continuing medical education for physicians. The Johns Hopkins University designates this continuing medical education activity for up to 24 credit hours in Category 1 of the Physician's Recognition Award of the American Medical Association. Postmarked prior to August 1, 1997, $495 - Physicians; $250 - Residents and Fellows, with letter from department chairperson verifying status; $535 - Physicians; $285 - Residents and Fellows after August 1, 1997.

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**Keywords:**

Diarrhea; Gut-microflora; Lactobacillus reuteri; Rotavirus

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