Effects of *Lactobacillus rhamnosus* Strain GG in Pediatric Obesity-related Liver Disease

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ABSTRACT

Objective: Various lines of evidence suggest that malfunctioning of the gut–liver axis contributes to hepatic damage of rodents and humans with nonalcoholic fatty liver disease. We evaluated the effects of short-term probiotic treatment in children with obesity-related liver disease who were noncompliant with lifestyle interventions.

Patients and Methods: Twenty obese children (age 10.7 ± 2.1 years) with persisting hypertransaminasemia and ultrasonographic (US) bright liver were enrolled in this double-blind, placebo-controlled pilot study. At baseline, patients underwent clinical and laboratory anthropometric evaluation, measurement of the US hepatorenal ratio, standard liver function tests, oral glucose tolerance test, serum tumor necrosis factor-alpha, the glucose hydrogen breath test, and evaluation of serum antibodies to antipeptidoglycan-polysaccharide polymers. After exclusion of causes of liver disease other than obesity, patients received either probiotic *Lactobacillus rhamnosus* strain GG (12 billion CFU/day) or placebo for 8 weeks.

Results: Multivariate analysis after probiotic treatment revealed a significant decrease in alanine aminotransferase (average variation vs placebo $P = 0.03$) and in antipeptidoglycan-polysaccharide antibodies (average variation vs placebo $P = 0.03$) irrespective of changes in BMI z score and visceral fat. Tumor necrosis factor-alpha, and US bright liver parameters remained fairly stable.

Conclusions: Probiotic *L rhamnosus* strain GG warrants consideration as a therapeutic tool to treat hypertransaminasemia in hepatopathic obese children noncompliant with lifestyle interventions.

Key Words: children, *Lactobacillus* GG, liver, nonalcoholic fatty liver disease, probiotics

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Nonalcoholic fatty liver disease (NAFLD) is a worldwide medical problem that parallels the obesity epidemic (1) and has become the most common liver disorder at all ages (2,3). Loss of weight and regular physical activity are the mainstay of treatment, but these are difficult to achieve (4–7). Although histological liver abnormalities are not generally a major concern in children, treatment is essential to avoid progression to more severe forms during or after transition into adult age (7,8). However, no proven effective pharmacological therapies are yet available (9,10).

Several studies have suggested that small intestinal bacterial overgrowth (SIBO) may contribute to the pathogenesis of NAFLD (11–13). Manipulation of intestinal flora with probiotics in experimental NAFLD of rodent animal models did indeed have a beneficial effect (14,15). In humans, a preliminary study investigating the outcome of probiotic VSL#3 (a mixture of 4 lactobacilli strains) therapy on liver dysfunction parameters in different categories of adult chronic liver disease including NAFLD also yielded promising results (16); however, the opposite results were reported in a recent article (17). The aim of our pilot study was, therefore, to evaluate whether a short course of therapy with probiotics improves the outcome of liver function tests in children with obesity-related liver disease.

PATIENTS AND METHODS

Obese children followed up at our pediatric obesity and pediatric liver units, with a body mass index (BMI) > 95th percentile for age and sex, who failed to adhere to previous slimming diets and who had not undergone previous pharmacological treatment for obesity, were offered the possibility of entering the present study. Twenty patients with persistent (>3 months) liver abnormalities (alanine aminotransferases [ALT] higher than upper normal values [40 U/L] associated with ultrasonographic [US] liver brightness suggestive of fatty liver) were enrolled.

Exclusion criteria were the coexistence of causes of increased transaminase levels other than obesity (namely muscular disease, viral hepatitis B and C, autoimmune hepatitis, α_1-antitrypsin deficiency, cystic fibrosis, Wilson disease, hemochromatosis, hereditary fructose intolerance, amino acid disorders, atypical celiac disease, alcohol abuse, and drug toxicity), which were investigated by appropriate biochemical tests or verified by anamnestic data. Patients receiving concomitant antibiotic treatment were also excluded.

At entry, weight, height, BMI, BMI z score, and visceral fat thickness measured by US (18) were investigated. Standard liver function tests and US brightness were evaluated and graded as described elsewhere (4,19). A quantitative ratio of liver versus renal brightness that referred to specific regions of interest was obtained according to Mancini et al (20). All of the patients underwent a standard oral glucose tolerance test. The glucose H_2 breath test (H2BT) was performed after an overnight fast (1 g/kg, maximum 50 g/250 mL) (21). Patients were asked to avoid foods containing beans, bran, and high-fiber cereals on the day before the test. Breath

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samples were collected every 15 minutes for 2 hours. The test was considered positive for SIBO if the fasting breath H_2 concentration was > 20 ppm and/or increased >10 ppm above baseline within the first 2 hours. Serum levels of tumor necrosis factor-alpha (TNF-α) were measured by quantitative sandwich enzyme immunoassay technique (R&D Systems, Minneapolis, MN). Serum antibodies to peptidoglycan-polysaccharide (PG-PS) complexes were determined by classical enzyme-linked immunosorbent assay according to the method of Hazenberg et al (22) and using commercially available PG-PS 100 P (Fluka Kemie GmbH, Buchs, Switzerland) (23). In the case of the hepatorenal US ratio, TNF-α, and PG-PS IgA, we compared the values in 20 age-, sex-, and weight-matched obese nonhepatopathic children.

The 20 children were randomly allocated to 1 of the 2 groups for a pilot randomized, double-blind (sealed envelopes), placebo-controlled study. The study drugs were oral *Lactobacillus* GG (12 billion CFU/day) and an indistinguishable placebo administered for 8 weeks. The primary outcome measure was improvement and/or normalization of ALT levels (<40 IU/L). Secondary endpoints were treatment-related changes in liver echogenicity, TNF-α values, and, possibly, intestinal tests (H2BT and PG-PS antibodies).

Changes in lifestyle were not encouraged during the brief framework of the study. Parents were instructed to report any adverse effect that occurred during treatment and to supervise the administration of pills. Parents gave written informed consent. The study was conducted in accordance with Good Clinical Practices and the Declaration of Helsinki, and was approved by the ethics committee of our university hospital (number 8404).

Statistical Analysis

Continuous variables are reported as mean ± standard deviation. Two-tailed tests of significance are reported, and $P < 0.05$ were considered statistically significant. The unpaired Student *t* test was used to assess differences in the average variations (8 weeks–baseline) between the treatment and placebo groups, as well as between the baseline means of the 2 groups. The possible confounding effect of BMI *z* score variations on ALT concentrations was explored by a multiple regression model with ALT as dependent variable and with BMI *z* score variation and treatment as independent variables. The Fisher exact test was used to calculate an exact probability value for the relation between 2 dichotomous variables.

RESULTS

Demographic data, anthropometric measurements, and biochemical and ultrasonographic features in the 20 patients at entry into the study are shown in Table 1. Baseline results confirmed that isolated hypertransaminasemia was the only abnormality to emerge from the liver tests. All of the children had mild to moderate US fatty liver. Diabetes was excluded in all of the children based on the results of oral glucose tolerance test. H2BT was abnormal (H_2 peak of 19 ppm above baseline at 60 minutes) only in 1 patient (of the treated group).

All of the patients completed the 8-week study with good adherence to therapy as documented by pill count and parents' supervision reports. No adverse effects were reported. Anthropometric, laboratory, and instrumental findings in the 2 groups of patients at baseline and after 8 weeks of probiotic or placebo treatment are listed in Table 2. As depicted also in Figure 1, the average variations of ALT levels in the 2 arms of the study were significantly more pronounced in the *Lactobacillus* GG–treated group ($P = 0.03$). Eight of the 10 treated patients and 3 of 10 patients in the placebo group attained ALT values <40 U/L, but the difference did not reach statistical significance ($P = 0.069$ Fisher exact test). PG-PS IgA significantly decreased only in the probiotic-treated group. Anthropometric data including US visceral fat, TNF-α, and US liver brightness evaluated by the hepatorenal ratio remained fairly stable in both groups during the study (Table 2).

TABLE 1. Demographic data, anthropometric measurements, and biochemical and ultrasonographic features in the 20 hepatopathic obese children at study entry

	Patients, n = 20
Sex, M/F	18/2
Age, y	10.7 ± 2.1
Weight, kg	61.7 ± 12.7
Height, cm	148.0 ± 10.0
BMI *z* score	2.2 ± 0.27
US visceral fat, mm	11.4 ± 2.9
ALT, IU/L (normal values < 40)	66.9 ± 27.3
AST, IU/L (normal values < 40)	41.6 ± 16.54
Triglycerides, mg/dL	96.85 ± 9.7
Cholesterol, mg/dL	157.0 ± 29.9
PG-PS IgA, OD*	0.50 ± 0.20†
TNF-α, pg/mL*	8.04 ± 5.0‡
Hepatorenal US ratio*	1.24 ± 0.21§

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; IgA = immunoglobulin A; OD = optical density; PG-PS = peptidoglycan-polysaccharide; TNF-α = tumor necrosis factor-alpha; US = ultrasonographic.

* Normal values in 20 age-, sex-, and weight-matched nonhepatopathic obese children: †0.33 ± 0.11 ($P < 0.008$); ‡7.27 ± 3.11 (P = NS); §1.10 ± 0.11 ($P = 0.003$).

DISCUSSION

This is the first study to investigate the effects of probiotic treatment on obesity-related liver abnormalities in children. The study was designed as a short-term pilot trial to circumvent the frequently reported unpredictable effects of lifestyle changes that usually confound the results of a long-term study in this population. In fact, even minimal weight and lifestyle changes may affect the biochemical parameters of NAFLD (4–6). Here we show that a short course of probiotic treatment significantly improved ALT values, which in most cases became normal, and that this effect was independent of weight changes.

Small intestinal bacterial overgrowth has been implicated in the pathogenesis of NAFLD (11–13). The results of the glucose H2BT, which is a surrogate test for SIBO evaluation, in our children did not confirm the increased prevalence of SIBO reported in morbidly obese adults (12). However, the level of PG-PS IgA antibodies, another surrogate for SIBO, was significantly higher than that of a group of 20 age-, sex-, and weight-matched obese nonhepatopathic controls (Table 1), and moreover they significantly decreased only in the children treated with probiotics and not in the placebo-treated group. Because PG-PS polymers are integral cell wall components of most bacterial species, including intestinal Bacteroides, which are the major determinants of SIBO (24,25), the decrease of PG-PS IgA antibodies after treatment may imply abnormalities in the patients' small intestinal microflora. Wigg et al (11) found that levels of endotoxin (ie, another SIBO marker) did not differ between patients with NAFLD and healthy controls. In a more recent work, endotoxin levels in the peripheral circulation were increased in patients with NAFLD, but they were unrelated to disease severity (26). Given the differences between

TABLE 2. Anthropometric, laboratory, biochemical, and instrumental findings in the 2 groups of children with obesity-related liver disease at baseline (T0) and after 8 weeks of probiotic or placebo treatment (T8)

	Controls (placebo)		Probiotic treatment			
	T0 baseline	T8 weeks	T0 baseline	T8 weeks	Average variation (probiotic vs placebo)	Baseline *t* test
ALT, IU/L	63.6 ± 18.47	61.6 ± 31.80	70.3 ± 34.76	40.1 ± 22.37	0.03	NS
BMI *z* score	2.12 ± 0.24	2.00 ± 0.26	2.29 ± 0.30	2.21 ± 0.31	NS	NS
Visceral fat, mm	10.79 ± 3.29	11.39 ± 2.35	12.04 ± 2.58	14.04 ± 5.14	NS	NS
TNF-α, pg/mL	8.28 ± 5.11	6.88 ± 6.43	7.74 ± 5.28	5.42 ± 2.10	NS	NS
Hepatorenal US ratio	1.31 ± 0.26	1.30 ± 0.15	1.17 ± 0.12	1.22 ± 0.12	NS	NS
PG-PS IgA, OD	0.64 ± 0.32	0.68 ± 0.36	0.68 ± 0.26	0.60 ± 0.25	0.03	NS

ALT = alanine aminotransferase; BMI = body mass index; IgA = immunoglobulin A; NS = nonsignificant; OD = optical density; PG-PS = peptidoglycan-polysaccharide; TNF-α = tumor necrosis factor-alpha; US = ultrasonographic.

peripheral and portal blood values and potential methodological difficulties caused by the short half-life of endotoxins, data obtained from the measurement of serum endotoxin levels should be interpreted with caution (11,26). For this reason, we did not measure endotoxin levels in the present study.

TNF-α is believed to be one of the protagonists of the inflammatory response in NAFLD after stimulation via Toll-like receptors by endotoxins, which reach the liver via portal flow through a more permeable intestinal barrier. However, similar to a previous study (11), the TNF-α values in our hepatopathic obese patients overlapped those of the 20 age- and weight-paired obese individuals. They tended toward a mild decrease in both treatment arms without, however, reaching statistical significance. *Lactobacillus* GG–induced modulation on studied parameters involved in NAFLD pathogenesis will probably require long-term studies. The effects of probiotics on NAFLD have been evaluated in animal models and, although to a lesser extent, in human adults. Probiotic VSL#3 treatment in NAFLD ob/ob mice lowered the transaminase level and affected histological liver inflammation without modifying steatosis (14). The same treatment in mice with diet-induced NAFLD attenuated fibrosis without affecting steatohepatitis (15,27). The unchanged US hepatorenal ratios in our patients are in line with these data. Another study showed that probiotics had a beneficial effect on several types of human liver disease including NAFLD, probably acting on the steatosis component (16). Contrary to what may be expected from the results of animal studies, a recent preliminary report on human NAFLD found a reversible increase in magnetic resonance imaging–calculated fat liver content during probiotic treatment (17). However, details of possible simultaneous weight changes were not reported. Given the above, individuals enrolled in such studies should undergo accurate anthropometric monitoring to ensure that other factors do not confound the effect of treatment on the outcome.

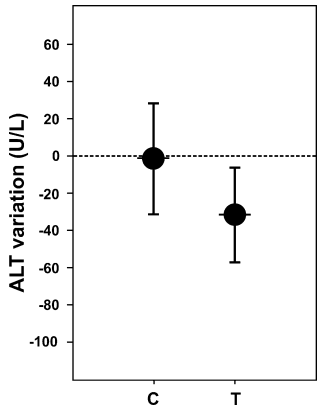

FIGURE 1. Effects of treatment on serum alanine aminotransferases (ALT) values in patients receiving either placebo (placebo controls, C) or *Lactobacillus* GG (T). $P = 0.03$.

Our results do not shed light on the mechanism(s) underlying the improvement of our patients' ALT values. Recent evidence indicates that there are quantitative (ie, SIBO) and qualitative differences in gut microbiota and microbiome between lean and obese individuals (28). The fact that our patients were completely asymptomatic from a gastrointestinal viewpoint may argue against the existence of a true SIBO. Because positive H2BTs in the exceedingly large percentage of Wiggs' patient series has been questioned by several investigators (29), it is feasible that in our study, *Lactobacillus* GG exerted a beneficial effect on obesity-related liver disease by modulating an altered bacterial composition rather than reducing its quantity (30). However, methodological aspects may have affected the results of the glucose H2BT in detecting SIBO; namely, the dose of the sugar or the duration of the test may not have been sufficient to exclude a bacterial contamination restricted to the distal small bowel. (21) Future large-scale intervention trials investigating the potential benefit of probiotics should include a combination of gastrointestinal dysfunction tests, including lactulose H2BT and intestinal permeability studies.

In view of the excellent tolerance and the remarkable reduction of transaminase after probiotics in our double-blind, placebo-controlled, short-term pilot study, *Lactobacillus rhamnosus* strain GG could be considered a potential therapeutic tool for pediatric obesity-related liver disease in children who are unable to lose weight. Our promising data provide a rationale for starting a larger study on possibly biopsy-proven pediatric NAFLD.

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