Beneficial Effects of a Probiotic VSL#3 on Parameters of Liver Dysfunction in Chronic Liver Diseases

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Objectives: To evaluate whether chronic therapy with probiotics affects plasma levels of cytokines and oxidative/nitrosative stress parameters, as well as liver damage, in patients with various types of chronic liver disease.

Patients and Methods: A total of 22 nonalcoholic fatty liver disease (NAFLD) and 20 alcoholic liver cirrhosis (AC) patients were enrolled in the study and compared with 36 HCV- positive patients with chronic hepatitis without (20, CH) or with (16, CC) liver cirrhosis. All patients were treated with the probiotic VSL#3. Routine liver tests, plasma levels of tumor necrosis factor alpha (TNF-α), interleukin (IL)-6 and -10, malondialdehyde (MDA), and 4-hydroxynonenal (4-HNE), S-nitrosothiols (S-NO), were evaluated on days −30, 0, 90, and 120.

Results: Treatment with VSL#3 exerted different effects in the various groups of patients: in NAFLD and AC groups, it significantly improved plasma levels of MDA and 4-HNE, whereas cytokines (TNF-α, IL-6, and IL-10) improved only in AC patients. No such effects were observed in HCV patients. Routine liver damage tests and plasma S-NO levels were improved at the end of treatment in all groups.

Conclusions: Results of the study suggest that manipulation of intestinal flora should be taken into consideration as possible adjunctive therapy in some types of chronic liver disease.

Key Words: liver disease, gut-liver axis, bacteria strain

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Numerous pharmaceutical preparations, using various strains of bacteria, are currently available and, indeed, these "functional foods" or "new drugs" have been proposed in the management of patients with *Helicobacter pylori* infection and in inflammatory bowel disease, either alone or associated with conventional therapy.[1] Few sporadic data have been reported on the use of these products in patients with chronic liver disease.[2–4] The liver continuously receives blood from the gut through the portal system; therefore, there is a close and constant relationship between gut and liver.[5] Animal studies have shown that translocation of bacterial products from the intestinal lumen to the mesenteric circulation and the lymphatics activates the Kupffer cells in the liver, induces regional and systemic production of proinflammatory cytokines, enhances production of free radical species, and activates nitric oxide (NO)-synthase in the splanchnic area. Intestinal bacteria produce ethanol and acetaldehyde. Thus, gut-derived endotoxins and active metabolites may both contribute to the evolution of alcohol- or obesity-related liver steatosis to steatohepatitis and fibrosis, as well as to the onset of portal hypertension.[6–11] Treatment with oral antibiotics that are poorly absorbed or lactobacilli inhibits the shift of steatosis to steatohepatitis in animals with obesity or animals fed with ethanol and improves the hemodynamics of portal circulation in patients with portal hypertension.[2–4] As is well known, the treatment of hepatic encephalopathy is based on the modification both of the composition and the metabolism of gut microflora,[12] and our group has demonstrated that chronic treatment with a mixture of different bacterial strains leads to an improvement in this syndrome in cirrhotics.[13,14]

The present open pilot study was carried out to evaluate the effects of chronic treatment with a mixture containing 450 billion bacteria in different strains (VSL#3) in patients with chronic liver damage. This pharmaceutical preparation has demonstrated to modify intestinal microflora in humans[15,16] and lead to an improvement in nonalcoholic fatty liver disease (NAFLD) in experimental animals.[3] Four groups of patients have been compared, classified according to the etiology and entity of the liver disease into patients with chronic hepatitis [NAFLD and hepatitis C virus (HCV)-related chronic hepatitis] and patients with cirrhosis (alcohol- or HCV-related). The following parameters were taken into consideration: 1) markers of liver damage, 2) plasma levels of cytokines, and 3) markers of enhanced production of oxygen and NO-related free radicals.

PATIENTS AND METHODS

The study protocol was approved by the Ethics Committee of the Department, and written informed consent was obtained from each patient prior to the study. Enrolled in the study were 78 patients: 22 with biopsy-proven NAFLD, 20

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with alcoholic cirrhosis (group AC), 20 with biopsy-proven HCV-related chronic hepatitis (group CH), and 16 with HCV-related cirrhosis (group CC). The diagnosis of cirrhosis was based on biochemical, ultrasonographic, and endoscopic findings, and the degree of liver disease was Child A.[17] None of the patients was on a particular diet during the study. All patients were submitted to clinical and biochemical evaluation 1 month prior to enrollment; thereafter, they were treated for 3 months with VSL#3 (VSL Pharmaceuticals, Inc, Ft. Lauderdale, FL), a mixture containing 450 billion bacteria in various strains (*Streptococcus thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus bulgaricus*) (2 × 2/die), followed by another month of washout. On days −30, 0, 90, and 120 days, venous samples were a drawn after overnight fasting to determine:

1. Routine liver tests
2. Plasma levels of:
   a) Cytokines tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-10 (Milenia Biotech, Germany). Selection of these cytokines was based on data in the literature concerning their involvement and plasma variations in different types of chronic liver disease.[7]
   b) Markers of lipid peroxidation: malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), using commercial kits (LPO Oxis International, Portland, OR).[18]
3. S-Nitrosothiols (S-NO). This parameter was selected here since, in our experience and that of others, it should indicate the circulating amount, as well as the metabolism of NO.[19,20] S-NO was determined by the spectrophotometric method using nitrosoglutathione (GSNO) (Sigma-Aldrich, Poole, UK) as standard.[21] Values were expressed as μmol/L.

Statistical Analysis

Data were recorded both on paper and in an electronic database. Results were expressed as median and range, and as number (percentage) of patients with a certain condition. The SPSS program 11.0 was used for the statistical analyses. The analysis of variance and Student's *t* test, for normally distributed variables, and Mann-Whitney *U* test, for not normally distributed variables, were used to evaluate the differences between data and groups. Differences in frequency were calculated by c^2. A value of $P \leq 0.05$ was considered significant.

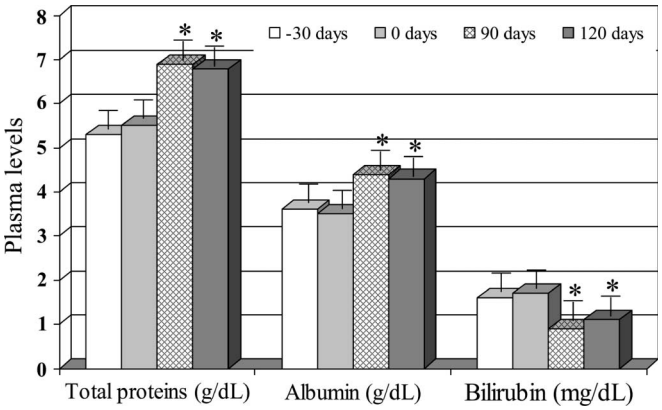

FIGURE 2. Plasma levels of total proteins (g/dL), albumin (g/dL), and bilirubin (mg/dL) (mean ± SD) in AC patients both in basal conditions and after VSL#3 treatment (*$P < 0.01$ vs. 0).

RESULTS

The final study groups consisted of the following: NAFLD group: M:F 17:5, median age 37 years, range 29 to 56 years, no alcohol or drug users, not previously treated with any drug; AC group: M:F 16:4, median age 50 years, range 38 to 54 years, median daily alcohol intake 80 g, range 60 to 220 g; CH group: all nonresponders to previous treatment with interferon + ribavirin, withdrawn at least 6 months (range, 6 months to 2 years) prior to the present study, none of whom alcohol users, M:F 12:8, median age 52 years, range 40 to 59 years; CC group: M:F 7:9, median age 59 years, range 51 to 68 years. These patients had not received pharmacological treatment, and alcoholics had stopped alcohol intake for almost 6 months prior to the study. This was confirmed by

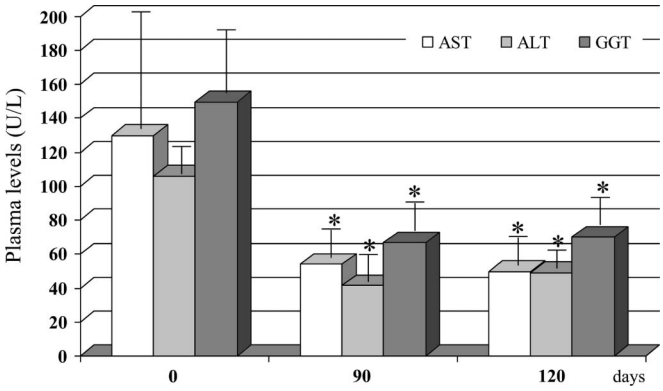

FIGURE 1. Plasma levels of aspartate aminotransferase (U/L), alanine aminotransferase (U/L), and gamma-glutamyltranspeptidase (U/L) (mean ± SD) in AC patients both in basal conditions and after VSL#3 treatment (*$P < 0.01$ vs. 0).

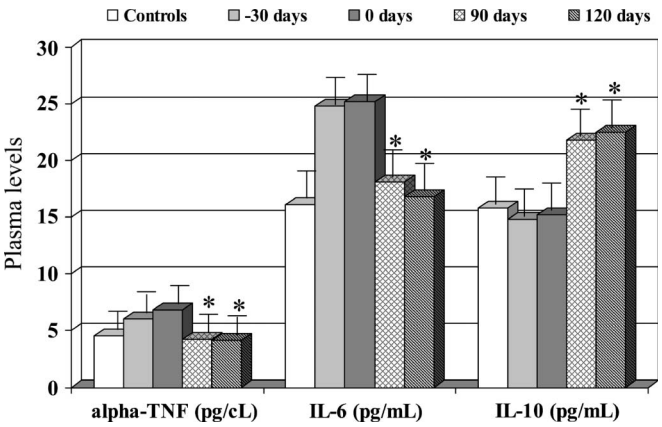

FIGURE 3. Plasma levels of TNF-α (pg/cL), IL-6 (pg/mL), and IL-10 (pg/mL) (mean ± SD) at −30 and 0 time, after VSL#3 treatment and washout in AC patients (*$P < 0.01$ vs. controls, −30 and 0 time).

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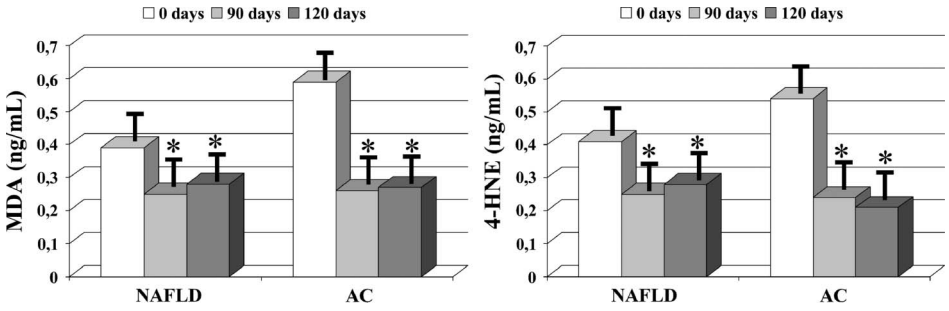

FIGURE 4. Effect of VSL#3 treatment on plasma levels of MDA and 4-HNE (mean ± SD; ng/mL) in NAFLD and AC patient groups (*$P < 0.01$ vs. 0 days) (MDA normal values 0.11 ± 0.04 ng/mL; 4-HNE normal values 0.13 ± 0.03 ng/mL). Data from other groups are not shown since they did not differ from normal values either in basal conditions or after treatment.

standardized tests.[22] There were four dropouts (2 in the CH group, 1 in NAFLD, 1 in CC) because of drug intolerance. Plasma levels of liver enzymes, aspartate aminotransferase and alanine aminotransferase, improved significantly in all patients after treatment with VSL#3, with the effect being maintained after washout in the CH, NAFLD, and AC groups; in these latter patients, gamma-glutamyltranspeptidase levels were also significantly and persistently improved. Data from AC patients are summarized in Figure 1. None of the patients in these groups showed any change in body mass index at the end of the study, compared with that in basal conditions. In alcoholic cirrhotics, a significant variation in total proteins albumin and bilirubin was found at 90 and 120 days (Figure 2). Plasma levels of TNF-α, IL-6, and IL-10 were significantly impaired, with respect to normal values, only in AC in basal conditions. In this group, these three cytokines returned to within normal limits following treatment with VSL#3 (Figure 3). As reported by us and others,[18,23] MDA and 4-HNE, which were significantly increased only in the NAFLD and AC groups compared with normal values, dropped significantly after treatment with VSL#3 (Figure 4). Values of S-NO were significantly increased in all groups at baseline (normal values, 7.4 ± 1.5 μmol/L), being more marked in NAFLD and AC patients. Treatment with VSL#3 led to a significant decrease in S-NO levels in all groups, which, furthermore, persisted even during the washout period (Figure 5).

DISCUSSION AND CONCLUSIONS

Results emerging from the present investigation demonstrate that manipulation of the intestinal flora induces a significant beneficial effect in patients with various types and degree of chronic liver disease. Data are in keeping with the results of our previous study,[24] which showed that the combination of two mixtures of bacteria strains (both containing various bacteria strains such as *S. salivarius, L. bifidus, L. acidophilus, L. plantarum, L. casei, L. bulgaricus*) led to an improvement in liver function both in patients with AC and NAFLD.

The present study was designed to evaluate the effect of another pharmaceutical preparation with a bacterial strain, VSL#3, on plasma levels of cytokines and of markers of oxidative-nitrosative stress in patients with NAFLD and alcoholic liver disease, comparing findings with with those in HCV-positive patients. Indeed, in NAFLD and alcoholics, intestinal microflora contributes to the onset and progression of chronic liver damage by way of translocation of endotoxins from the intestinal lumen to the mesenteric circulation and its lymphatics and through the direct production of ethanol and acetaldehyde in the lumen following fermentation of dietary carbohydrates. Endotoxins activate Kupffer cells in the liver and enhance the production of TNF-α and IL-6. These cytokines are involved in the synthesis of acute-phase proteins and activate the production of TGF-β, which contributes to the onset of liver fibrosis. The increase in pro-inflammatory cytokines leads to a decrease in the antinflammatory and hepatoprotective cytokines, such as IL-10.[25–28] Endotoxins also induce the production of reactive oxygen intermediates, such as H_2O_2 and NO, and activate redox-sensitive transcription factors in hepatocytes and liver macrophages. A very large number of reports in the literature have shown that oxidative and nitrosative stress are both pathways involved in the pathophysiology of alcoholic and nonalcoholic chronic liver damage, and in patients with alcoholic and nonalcoholic steatohepatitis, plasma levels of TNF-α and IL-6 are increased with a significant correlation between serum concentrations of these cytokines and severity of the liver involvement[18,29,30]; lipid peroxidation markers are enhanced and antioxidants are decreased.[18,29,30]

Lactic acid bacteria have been an integral component of the human diet for centuries. Colonization of the gastrointestinal tract, by these probiotics, results in a modification of gut

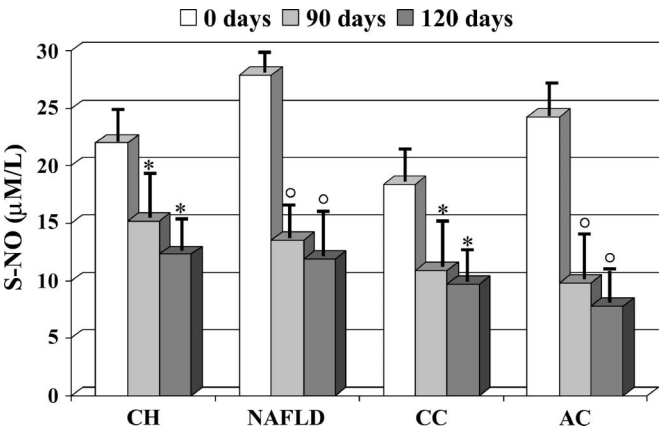

FIGURE 5. Plasma levels of S-NO (mean ± SD; μmol/L) in basal conditions, after VSL#3 treatment and after washout in the various groups of patients. Compared with normal values (7.4 ± 1.5 μmol/L), S-NO were significantly increased in basal conditions in all groups; VSL#3 treatment constantly improved S-NO levels (°$P < 0.01$ and *$P < 0.05$ vs. 0 days).

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flora and reduces the proinflammatory species.[31–33] In animals with alcoholic and nonalcoholic steatohepatitis, treatments that inhibit Kupffer cell activation, with poorly absorbed antibiotics or metronidazole, or with lactobacilli, including VSL#3, have a beneficial effect upon liver damage.[3,4,31–34] The VSL#3 preparation under study has been shown to modify intestinal microflora in humans,[15,16] and, in the present investigation, persistence of the beneficial effects was confirmed also during the follow-up period. Indeed, this treatment significantly reduced plasma levels of oxidative/nitrosative stress and restored cytokine levels in those patients presenting alterations in these parameters in basal conditions. Moreover, plasma levels of liver damage and function significantly improved, also in HCV-positive patients and in alcoholics.

These preliminary data suggest the need for well-controlled trials. Indeed, the effects on liver tests and on some parameters of pathophysiologic events related to liver damage, the low cost, the good tolerability in the majority of cases, would appear to indicate probiotics almost as a complementary therapeutic approach in liver disease patients.

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