Synbiotic Modulation of Gut Flora: Effect on Minimal Hepatic Encephalopathy in Patients With Cirrhosis

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Minimal hepatic encephalopathy (MHE) is an important disorder that may seriously impair daily functioning and quality of life in patients with cirrhosis. Treatment with lactulose is of benefit. The possible role of synbiotics (probiotics and fermentable fiber) has not been assessed. We screened 97 consecutive cirrhotic patients without overt hepatic encephalopathy for MHE using the number connection test and measurement of brainstem auditory evoked potentials. MHE, defined by abnormality on at least one test modality, was present in 58 (60%) patients. Fifty-five of these patients with MHE were randomized to receive a synbiotic preparation (n = 20), fermentable fiber alone (n = 20), or placebo (n = 15) for 30 days. Cirrhotic patients with MHE were found to have substantial derangements in the gut microecology, with significant fecal overgrowth of potentially pathogenic *Escherichia coli* and *Staphylococcal* species. Synbiotic treatment significantly increased the fecal content of non-urease-producing *Lactobacillus* species at the expense of these other bacterial species. Such modulation of the gut flora was associated with a significant reduction in blood ammonia levels and reversal of MHE in 50% of patients. Synbiotic treatment was also associated with a significant reduction in endotoxemia. The Child-Turcotte-Pugh functional class improved in nearly 50% of cases. Treatment with fermentable fiber alone was also of benefit in a substantial proportion of patients. In conclusion, treatment with synbiotics or fermentable fiber is an alternative to lactulose for the management of MHE in patients with cirrhosis. (HEPATOLOGY 2004;39:1441–1449.)

Minimal hepatic encephalopathy (MHE) in patients with liver cirrhosis is defined by the presence of otherwise unexplained cognitive abnormalities, only detectable on psychometric or neurophysiological testing, in the absence of overt hepatic encephalopathy (HE).[1] Increasing evidence indicates that MHE is an important disorder that may seriously impair a patient's daily functioning and quality of life.[2,3] Psychomotor slowing and deficits in attention, visual perception and visuoconstructive abilities are key features,[4,5] while fine motor performance is also impaired.[2,6] MHE may render a patient unfit to drive a motor vehicle[7] and is an important predictive factor for the development of overt HE.[8–10] MHE is present in 30% to 70% of cirrhotic patients without overt HE.[4,8,9,11–13] Neurophysiological measures, such as the brainstem auditory evoked potential (BAEP), may provide more objective results than psychometric tests, such as the number connection test (NCT), which can be influenced by age, education level, and learning effects.[1] Nonetheless, adequate psychometric testing has been shown to have greater diagnostic sensitivity for MHE than neurophysiological tests, at least in some series.[14,15] Use of a combination of test methods is recommended to most reliably diagnose MHE.[1]

Ammonia is a key factor in the pathogenesis of overt HE in cirrhotic patients,[16] and therapeutic interventions of proven benefit in this setting, such as treatment with lactulose (4-O-β-d-galactopyranosyl-d-fructose), are generally aimed at reducing ammonia levels.[17] Lactulose lowers the colonic pH as a result of the production of organic

Abbreviations: MHE, minimal hepatic encephalopathy; HE, hepatic encephalopathy; BAEP, brainstem auditory evoked potential; NCT, number connection test; spp, species; E. coli, Escherichia coli*; ALT, alanine aminotransferase.*

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acids by bacterial fermentation. The decrease in pH creates an environment that is both hostile to the survival of urease-producing gut flora, such as *Klebsiella* species (spp) and *Proteus* spp, and conducive to the growth of acid-resistant, non-urease-producing species, such as lactobacilli and bifidobacteria, resulting in reduced production of ammonia in the colonic lumen. In addition, acidification of colonic secretions reduces the absorption of ammonia by nonionic diffusion.[17]

Increasing evidence indicates that, as with overt HE, ammonia is a key factor in the pathogenesis of MHE.[18,19] Increases in blood-brain permeability to ammonia,[18] cerebral metabolic rate for ammonia,[18] and cerebral glutamine/glutamate signaling on magnetic resonance spectroscopy[19,20] have each been documented. Ammonia-induced alterations in cerebral blood flow and glucose metabolism have also been described.[21] Treatment with lactulose is of benefit in patients with MHE.[2,22,23] The possible efficacy of an alternative approach to modulating the gut microecology and acidifying the gut lumen for therapeutic gain in cirrhotic patients with MHE, namely treatment with synbiotics (probiotics and fermentable fiber), has not previously been studied. Our pilot, placebo-controlled study investigated this issue. We show that synbiotic treatment significantly modifies the gut flora and fecal pH, leading to a significant reduction in venous ammonia levels and reversal of MHE in 50% of patients. Treatment with fermentable fiber alone was also of benefit.

Materials and Methods

Patients. A total of 97 consecutive outpatients with hepatic cirrhosis but no overt HE were screened for MHE, as described below. Cirrhosis was diagnosed histologically in 69 out of 97 (71%) patients and on clinical and radiological grounds[24] in the remaining 28 (29%) patients, in whom biopsy was contraindicated by uncontrolled coagulopathy and/or uncontrolled ascites. Patient characteristics, including the etiology of cirrhosis and Child-Turcotte-Pugh class,[25] are listed in Table 1. Patients were considered to have alcohol-related cirrhosis if alcohol intake had been in excess of 80 g/day in men and 30 g/day in women for more than 5 years and if testing for viral, metabolic, and immune aetiologies was negative.[24] Only patients who had been abstinent from alcohol for at least 2 months, as corroborated by family members and/or caregivers, were included. Patients with histological features of alcoholic hepatitis were excluded. If liver histology was not available, patients in whom the serum gamma-glutamyl transpeptidase level fell during a 2-month period of observation prior to study entry were excluded to avoid inclusion of patients with possible alcoholic hepatitis, as previously described.[26] Exclusion criteria also included a history within the previous 6 weeks of factors that may have influenced gut flora and circulating endotoxin and ammonia levels, including infection, treatment with antibiotics, lactulose or immunomodulatory drugs, and gastrointestinal hemorrhage.[24] In addition to intercurrent infection and gastrointestinal hemorrhage, patients with other possible causes of reversible hepatic functional decompensation, such as drug-related hepatotoxicity and choledocholithiasis, were excluded. Patients with other known precipitants of HE, including renal impairment, electrolyte imbalance, and complicating hepatocellular carcinoma, were also excluded. No patient with cirrhosis related to hepatitis B virus or hepatitis C virus received antiviral treatment prior to or during the study.

Table 1. Patient Characteristics

	Total Group (n = 97)	With MHE* (n = 58)	Without MHE (n = 39)
Age (yr)	55 ± 11	56 ± 11	49 ± 10
Gender (M:F)	88:9	56:2	32:7
Etiology of cirrhosis (%)			
Hepatitis virus B or C	75 (77)	43 (74)	31 (79)
Alcohol	19 (20)	12 (21)	5 (13)
Other	3 (3)	3 (5)	3 (8)
Turcotte-Child-Pugh classification			
A	29 (30)	10 (17)	19 (49)
B/C	68 (70)	48 (83)	20 (51)

NOTE. Age expressed as mean ± SD.

*3 patients were subsequently excluded because of inability to comply with serial neurophysiological testing. MHE was significantly more prevalent in Child-Turcotte-Pugh class B or C patients (48/68; 71%) than in Child-Turcotte-Pugh class A counterparts (10/29; 34%) ($P = .001$; Fisher exact test).

Informed written consent was obtained from each subject. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the Research Ethics Committee of the Public Health Section of Beijing Youan Hospital, China.

Diagnosis of MHE. Patients were assessed for MHE using both psychometric (NCT) and neurophysiological (BAEP) modalities. The NCT has been demonstrated to have high reproducibility and predictive value for the subsequent development of overt HE in cirrhotic patients with MHE,[2,10] while the BAEP has been identified as the single neurophysiological investigation of choice in this setting.[27] In the NCT, which measures cognitive motor abilities, patients must connect numbers from 1 to 25 printed on paper as quickly as possible. The test score is the time required to complete the test, including the time needed to correct any errors. In this study, the NCT was considered abnormal when the time taken was greater

than the mean + 2 standard deviations of that in healthy controls matched for age and educational level, as previously published.[28]

The BAEP was measured with patients in the dorsal decubitus position with closed eyes in a semidarkened room. Both ears were stimulated alternatively, with the nonstimulated ear masked by a 65 decibel noise. Reception was measured by means of an electrode placed on the vertex. The peak latency III and V and interpeak latency III–V were determined. Latencies were considered abnormal when greater than the mean + 2 standard deviations of those in healthy control patients, as previously published.[28]

MHE was diagnosed if at least one of the NCT and BAEP was abnormal. MHE was found to be present in 58 out of 97 (60%) patients, including 10 out of 29 (34%) Child-Turcotte-Pugh class A patients and 48 out of 68 (71%) Child-Turcotte-Pugh class B or C patients ($P = .001$). Three MHE patients were excluded from further analysis because of inability to comply with serial BAEP testing. The remaining 55 patients, who formed the cohort further investigated as described below, included 33 (60%) with an abnormal BAEP and 26 (47%) with an abnormal NCT. Four patients (7%) demonstrated abnormalities on both tests (Table 1).

Response was defined by normalization of the abnormal test parameter. Patients with abnormalities of both the NCT and BAEP prior to therapy were classified as responders only if both parameters normalized following treatment.

Supplementation With the Synbiotic Preparation. Coded sachets containing the various study preparations (A, n = 20; B, n = 20; C, n = 15) were pooled. One sachet was randomly drawn from this pool for each patient at study entry. Patients drawn to receive treatment with sachets A (Group A) received oral supplementation with a synbiotic preparation consisting of 4 freeze-dried, non-urease-producing bacteria, namely *Pediacoccus pentoseceus* 5-33:3, *Leuconostoc mesenteroides* 32-77:1, *Lactobacillus paracasei* subspecies *paracasei* 19 and *Lactobacillus plantarum* 2592, each at a dose of 10^{10} colony forming units per sachet, along with 10 g of bioactive, fermentable fiber (beta glucan, 2.5 g; inulin, 2.5 g; pectin, 2.5 g; resistant starch, 2.5 g) (Cocktail 2000; Medipharm, Kagerod, Sweden). Patients drawn to receive treatment with sachets B (Group B) received the 4 bioactive, fermentable fibers (2.5 g of each, as mentioned previously) but not the lactic acid bacteria (Medipharm). Patients drawn to receive treatment with sachets C (Group C) received a placebo, namely a wheat-based, nonfermentable fiber (Medipharm).

Table 2. Clinical and Demographic Characteristics of Patients Randomized to Groups A, B, and C

	Group A (n = 20)	Group B (n = 20)	Group C (n = 15)
Age (yr)	55 ± 12	53 ± 10	57 ± 12
Gender (M:F)	20:0	19:1	14:1
Etiology of cirrhosis (%)			
Hepatitis B virus	14 (70)	14 (70)	11 (73)
Hepatitis C virus	0 (0)	1 (5)	1 (7)
Alcohol	4 (20)	4 (20)	3 (20)
Other	2 (10)	1 (5)	0 (0)
PBC	1 (5)	1 (5)	–
PSC	1 (5)	–	–
Turcotte-Child-Pugh classification			
A	3 (15)	3 (15)	2 (13)
B/C	17 (85)	17 (85)	13 (87)

NOTE. Age expressed as mean ± SD.

Abbreviations: PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

All patients in Groups A, B, and C received one sachet of the respective synbiotic, fermentable fiber only or placebo preparation daily, taken in 150–200 mL water, for 30 days. Patients in Groups A, B, and C were well-matched for clinical and demographic variables including age, gender, etiology of cirrhosis and Child-Turcotte-Pugh classification (Table 2).

Which of sachets A, B, and C contained the synbiotic, fermentable fiber and nonfermentable fiber preparations was unknown to the investigators until after the completion of the study and results had been analyzed, when the code was broken.

Patients were reassessed for MHE with both the NCT and BAEP, along with determination of the Child-Turcotte-Pugh class and the other parameters described below, on day 30 of supplementation. Parallel NCTs were used to eliminate any possible learning effect on post-supplementation performance.

Quantitative Bacteriological Analysis of Fecal Samples. Fecal samples for quantitative bacteriological culture were collected in sterile containers at 3 time points, namely at baseline, on day 30 of supplementation with the synbiotic, fermentable fiber or placebo preparations, as applicable, and 14 days following cessation of supplementation. Selective and nonselective bacteriological media were used to determine viable counts of a number of gut flora, including *Lactobacillus* spp and *Bifidobacterium* spp (nonpathogenic, non-urease-producing Gram-positive aerobes), *Staphylococcus* spp and *Enterococcus* spp (potentially pathogenic Gram-positive aerobes), *Escherichia coli (E. coli)* and *Pseudomonas* spp (potentially pathogenic Gram-negative aerobes) and *Fusobacterium* spp (potentially pathogenic Gram-negative

anaerobes). Media included Columbia blood agar (Sigma, St. Louis, MO) for growth of aerobes; Wilkins-Chalgren agar (Sigma) for facultative anaerobes; Rogosa agar (Oxoid, Basingstoke, Hampshire, UK) for *Lactobacillus* spp; blood azide agar (Oxoid) for *Enterococcus* spp and nutrient broth number 2 (Oxoid) for *E. coli*. Media for the determination of viable counts of aerobes were incubated at 37°C and in 10% CO_2 for 48 hours. Anaerobic cultures were incubated in Gas Pak jars (Anaero-Gen, Oxoid) at 37°C for 96 hours. Representative bacterial colonies were identified to the genus level on the basis of their Gram stain reaction and colonial and cellular morphologies. Bacterial concentrations were expressed as the logarithm of colony- forming units per gram of dry feces.

Twenty asymptomatic volunteers with no history of liver or intestinal disease, alcohol intake less than 20 g/day, normal liver function test results, and no predisposition to altered gut flora, as above, served as controls to determine normal ranges for quantitative culture of gut flora. These control subjects were age- and sex-matched proportionate to the overall study group of 55 patients with cirrhosis and MHE, since age and gender distributions were comparable in each of Groups A, B, and C.

Measurement of Fecal pH. Aliquots of feces were diluted with distilled water (10g/100 mL) and centrifuged at 5000*g* for 5 minutes. Fecal samples at baseline and on day 30 of supplementation were homogenized, and the pH was measured (Benchtop 420 pH Meter, Orion, Beverly, MA).

Measurement of Laboratory Components of the Child-Turcotte-Pugh Classification and Serum Alanine Aminotransferase (ALT) Level. Serum bilirubin and albumin levels along with the prothrombin time were measured using standard techniques in our laboratory. Prothrombin activity was determined by subtracting the control prothrombin time from the patient's prothrombin time, and dividing by the control value. The result was expressed as a percentage. The serum ALT value was measured by standard techniques as an index of hepatic necroinflammatory activity.

Measurement of Venous Ammonia Levels. Fasting venous ammonia levels were measured using the Menagent Ammonia Test Kit II (Menarini Diagnostic, Firenze, Italy), according to the manufacturer's instructions, at baseline and on day 30 of supplementation with the symbiotic, fermentable fiber or placebo preparations, as applicable. Sensitivity of the assay was 10 μmol/L.

Measurement of Serum Endotoxin Levels. Peripheral blood was drawn using pyrogen-free needles, syringes, and containers (Becton Dickinson, Singapore). Serum was separated at 4°C and stored at −70°C in pyrogen-free polyethylene cryotubes (Nunc, Rochester, NY) until analysis within 6 weeks of collection. Serum endotoxin levels were measured using the chromogenic limulus amoebocyte lysate assay (Shanghai Medical-Chemical Institute, Shanghai, China), according to the manufacturer's instructions, at baseline and on day 30 of supplementation with the synbiotic, fermentable fiber or placebo preparations, as applicable. Sensitivity of the assay was 3 pg/mL.

Statistical Analyses. Statistical analyses were performed using the paired sample Student *t* test, Fisher exact test, and multivariate regression analysis, as appropriate (Systat for Windows, version 5.02; Systat, Evanston IL). The probability level of $P < .05$ was set for statistical significance.

Results

Quantitative Bacteriological Analysis of Fecal Samples. Cirrhotic patients with MHE were found to have significant fecal overgrowth of *E. coli* and *Staphylococcus* spp. Supplementation with the synbiotic preparation for 30 days (Group A) led to significant reductions in viable counts of both of these overgrowth species, with viable counts falling to levels comparable to those in healthy controls. Viable counts of *Fusobacterium* spp were also reduced. Counts of non-urease-producing *Lactobacillus* spp were significantly increased, with these species becoming the predominant marker organisms in feces. No significant changes in viable counts of *Bifidobacterium* spp, *Pseudomonas* spp, or *Enterococcus* spp were documented. The significantly increased viable counts of *Lactobabacillus* spp and reduced counts of *E. coli* and *Staphylococcus* spp remained evident at reassessment 14 days after cessation of supplementation (Table 3).

Supplementation with fermentable fiber alone (Group B) was associated with both a significant increase in viable counts of non-urease-producing *Bifidobacterium* spp and significant reductions in counts of both *E. coli* and *Fusobacterium* spp. Overgrowth with *E. coli* was reversed. *Bifidobacterium* spp became the predominant marker organisms in feces. No significant changes in viable counts of *Lactobacillus* spp, *Staphylococcus* spp, *Pseudomonas* spp, or *Enterococcus* spp were documented. Reduced counts of *E. coli* remained evident at reassessment 14 days after cessation of supplementation (Table 3). Supplementation with the non-fermentable fiber (placebo) preparation (Group C) was associated with no significant change in viable counts of any of the gut flora assessed (Table 3). The synbiotic, fermentable fiber and placebo preparations were well-tolerated by all patients, with no reports of adverse side effects. In particular, no patient reported diar-

Table 3. Changes in Viable Counts of Fecal Flora, Expressed as Log_{10} (CFU/g dry feces), in Cirrhotic Patients With MHE Presupplementation and Postsupplementation With the Synbiotic Preparation (Group A), Fermentable Fiber Alone (Group B), and Placebo (Group C)

	Lactobacillus spp			*Bifidobacterium* spp		
Healthy controls	**7.4 ± 0.5**			**7.2 ± 0.7**		
Cirrhotic patients with MHE	**Group A**	**Group B**	**Group C**	**Group A**	**Group B**	**Group C**
Presupplementation	7.4 ± 0.5	7.2 ± 1.3	7.7 ± 1.5	6.6 ± 0.7	7.2 ± 0.7	6.9 ± 0.7
Day 30 of supplementation	9.8 ± 0.6*	7.4 ± 0.9	7.8 ± 1.0	7.1 ± 0.7	9.2 ± 0.3*	6.7 ± 1.2
Day 14 after cessation of supplementation	9.6 ± 0.3*	7.5 ± 0.8	7.4 ± 0.7	7.4 ± 0.8	7.7 ± 0.6	6.4 ± 0.6
	Escherichia coli			*Staphylococcus* spp		
Healthy controls	**7.3 ± 0.6****			**6.6 ± 1.7*****		
Cirrhotic patients with MHE	**Group A**	**Group B**	**Group C**	**Group A**	**Group B**	**Group C**
Presupplementation	10.3 ± 0.7	10.1 ± 0.4	10.4 ± 0.9	8.4 ± 1.6	8.0 ± 0.7	8.1 ± 1.5
Day 30 of supplementation	7.2 ± 1.4*	7.6 ± 0.8*	10.1 ± 0.5	6.6 ± 1.7*	7.1 ± 1.2	8.0 ± 1.3
Day 14 after cessation of supplementation	7.1 ± 0.4*	8.1 ± 0.9****	10.2 ± 0.6	6.8 ± 0.2*	8.0 ± 1.0	8.0 ± 1.0
	Fusobacterium spp			*Pseudomonas* spp		
Healthy controls	**6.2 ± 0.4**			**7.4 ± 0.3**		
Cirrhotic patients with MHE	**Group A**	**Group B**	**Group C**	**Group A**	**Group B**	**Group C**
Presupplementation	7.5 ± 0.4	7.2 ± 0.6	7.1 ± 1.5	7.6 ± 0.5	7.4 ± 0.3	7.1 ± 0.1
Day 30 of supplementation	6.2 ± 0.4****	4.3 ± 1.5*	6.9 ± 1.4	7.0 ± 2.3	7.0 ± 2.1	7.4 ± 2.3
Day 14 after cessation of supplementation	6.8 ± 0.4	8.0 ± 1.0	7.1 ± 0.6	7.4 ± 0.4	7.0 ± 1.3	7.4 ± 0.5

	Enterococcus spp		
Healthy controls	**6.3 ± 1.6**		
Cirrhotic patients with MHE	**Group A**	**Group B**	**Group C**
Presupplementation	5.9 ± 1.3	6.1 ± 1.7	5.4 ± 0.6
Day 30 of supplementation	6.4 ± 1.4	6.2 ± 1.0	5.8 ± 1.0
Day 14 after cessation of supplementation	6.5 ± 1.4	6.2 ± 1.2	6.4 ± 1.4

NOTE. Values expressed as mean ± SD.
*$P < .01$ compared to presupplementation values.
**$P < .001$ compared to values in cirrhotic patients with MHE (10.3 ± 0.7).
***$P < .01$ compared to values in cirrhotic patients with MHE (8.4 ± 1.6).
****$P < .05$ compared to presupplementation values.

rhea or abdominal pain or became noncompliant for other reasons.

Fecal pH. The fecal pH was >5.5 in 89.2% of cirrhotic patients with MHE prior to supplementation with the various symbiotic, fermentable fiber and placebo preparations. The proportions of patients with fecal pH ≤5.5 on day 30 of supplementation was significantly increased in both Group A and Group B, but not in Group C (Table 4).

Venous Ammonia Levels. Significant reductions in venous ammonia levels were found on day 30 of supplementation compared to presupplementation values in patients in Groups A and B, but not Group C (Table 5).

Serum Endotoxin Levels. Significant reductions in serum endotoxin levels were found on day 30 of supplementation compared to presupplementation values in patients in Groups A and B, but not Group C (Table 5).

MHE Status. MHE was reversed on day 30 of supplementation in 10 out of 20 (50%) Group A patients and 10 out of 20 (50%) Group B patients. These rates of reversal of MHE in Groups A and B were each significantly higher than those in Group C patients (2/15; 13%)

Table 4. Fecal pH in Cirrhotic Patients With MHE Presupplementation and Postsupplementation With the Synbiotic Preparation (Group A), Fermentable Fiber Alone (Group B), and Placebo (Group C)

	≤5.5	**>5.5**
Presupplementation (%)	6/55 (11)	49/55 (89)
Day 30 of supplementation (%)		
Group A	15/20 (75)*	5/20 (25)*
Group B	14/20 (70)*	6/20 (30)*
Group C	0/15 (0)	15/15 (100)

*$P < .0005$ compared to presupplementation percentages.

Table 5. Venous Ammonia and Serum Endotoxin Levels in Cirrhotic Patients With MHE Presupplementation and Postsupplementation With the Synbiotic Preparation (Group A), Fermentable Fiber Alone (Group B), and Placebo (Group C)

	Venous Ammonia (μmol/L)			Serum Endotoxin (pg/mL)		
	Group A	Group B	Group C	Group A	Group B	Group C
Presupplementation	60.5 ± 2.9	63.6 ± 3.9	60.5 ± 2.9	110.0 ± 14.3	112.1 ± 14.1	110.7 ± 14.1
Day 30 of supplementation	38.6 ± 3.9*	41.5 ± 5.2**	58.6 ± 3.9	83.3 ± 13.4***	68.5 ± 8.4**	105.7 ± 3.6

NOTE. Values expressed as mean ± SD.
*$P < .01$ compared to presupplementation.
**$P < .001$ compared to presupplementation.
***$P < .05$ compared to presupplementation.

($P = .03$). The breakdown of the tests for MHE that normalized in the various groups is listed in Table 6. No patient with MHE developed overt HE during the study period. On multivariate analysis, treatment with synbiotics or fermentable fiber alone (rather than placebo) but neither the etiology of cirrhosis nor the age of the patient was significantly associated with resolution of MHE during the study period (Table 7).

Child-Turcotte-Pugh Classification. Improvement in the Child-Turcotte-Pugh classification on day 30 of supplementation compared to baseline was documented in a significantly greater proportion of Group A than Group C patients; an increased rate of improvement in Group B compared with Group C patients did not reach statistical significance (Table 8). No instances of deterioration in Child-Turcotte-Pugh classification following supplementation with any of the various preparations were documented. Compared to pretreatment values, a significant reduction in the serum bilirubin level and significant increases in both the serum albumin level and prothrombin activity were documented in both Group A and Group B, but not in Group C. A significant reduction in the serum ALT level followed treatment in Groups A and B. No significant change in the ALT level occurred in Group C (Fig. 1).

Discussion

This study confirms the high prevalence of MHE in patients with cirrhosis, especially if Child-Turcotte-Pugh class B or C,[4,8,9,11–13] and, moreover, documents substantial derangements of the gut microecology in this group. In particular, cirrhotic patients with MHE were found to have significant fecal overgrowth with potentially pathogenic Gram-negative (*E. coli*) and Gram-positive (*Staphylococcus* spp) aerobic gut flora. Supplementation with the synbiotic preparation for 30 days led to significant reductions in viable counts of *E. coli* and *Staphylococus* spp, with reversal of overgrowth of these flora. A significant reduction in viable counts of *Fusobacterium* spp, potentially pathogenic anaerobic Gram-negative bacteria, was also documented. Treatment led to a significant increase in viable counts of non-urease-producing *Lactobacillus* spp, with these species becoming the predominant of the measured organisms in feces. The effects of supplementation with the synbiotic preparation on gut flora for 30 days were durable, with the significantly increased viable counts of *Lactobabacillus* spp and reduced counts of *E. coli* and *Staphylococcus* spp persisting at reassessment 14 days after cessation of supplementation.

Acidification of colonic secretions, as reflected by the fecal pH and due to metabolism of the fermentable fiber component of the synbiotic preparation to organic acids by colonic flora,[17] occurred in 75% of cases. Such a phenomenon favors the survival of relatively acid-resistant, non-urease-producing bacteria,[17] such as those probiotics included in our synbiotic regimen. Blood ammonia levels were significantly lowered in the synbiotic-treated group compared to baseline, with the mean value reduced by 36%, presumably as a consequence of reduced intestinal production of ammonia due to a shift in proportions of urease-positive and urease-negative colonic bacteria, reduced diffusion of any ammonia formed in the colon as a

Table 6. Normalization of the NCT and BAEP, and Rates of Reversal of MHE, Following Supplementation for 30 Days With the Synbiotic Preparation (Group A), Fermentable Fiber Alone (Group B), and Placebo (Group C)

	Number of Patients With Normalization of Tests for MHE			
	NCT	BAEP	NCT + BAEP	Reversal of MHE (%)
Group A	6	6	2	10/20 (50)*
Group B	6	4	0	10/20 (50)*
Group C	2	0	0	2/15 (13)

*$P = .03$ compared to reversal rate in Group C.

Table 7. Multivariate Analysis of Factors Possibly Associated With Resolution of MHE During the Study Period

	r	P
Treatment with synbiotics or fermentable fiber (rather than placebo)	0.30	.03
Etiology of cirrhosis	−0.07	.59
Patient age (yr)	−0.21	.11

Table 8. Rates of Improvement in Child-Turcotte-Pugh Classification in Class B or C Patients at Initial Assessment, Following Supplementation for 30 Days With the Synbiotic Preparation (Group A), Fermentable Fiber Alone (Group B), and Placebo (Group C)

	Improvement in Child-Turcotte-Pugh Class (%)
Group A	8/17 (47)*,†
Group B	5/17 (29)‡
Group C	1/13 (8)§

*P = .04 compared to Group C.

†Including 2 patients who improved from class C to class B, and 6 patients who improved from class B to class A.

‡Including 1 patient who improved from class C to class B, and 4 patients who improved from class B to class A.

§This patient improved from class C to class B.

result of acidification, or a combination of these mechanisms. Increased hepatic metabolism of portal venous ammonia consequent to an improvement in liver function associated with synbiotic treatment, as discussed below, may also have been contributory. MHE was reversed in 50% of patients treated with synbiotics for 30 days, a response rate significantly higher than the 13% demonstrated in placebo-treated patients in our study, in whom no significant change in blood ammonia levels occurred, and comparable to that previously reported in patients treated with lactulose for 8 weeks.[23]

Treatment with fermentable fiber alone was also of benefit in cirrhotic patients with MHE. As with synbiotic-treated patients, the fecal pH was reduced in most cases. This was associated with both a significant increase in viable counts of non-urease-producing *Bifidobacterium* spp and significant reductions in counts of both aerobic (*E. coli*) and anaerobic (*Fusobacterium* spp) Gram-negative flora. Overgrowth of *E. coli* was reversed. *Bifidobacterium* spp became the predominant of the measured organisms in feces. The blood ammonia level was significantly reduced compared to pretreatment values, with the mean value falling by 35%. MHE resolved in 50% of patients, a proportion that, as in patients treated with synbiotics, was significantly higher than that in the placebo-treated group. Multivariate analysis indicated that instances of resolution of MHE that occurred in association with treatment with synbiotics or fermentable fiber alone were the consequence of these treatments and not influenced by potentially confounding factors such as the age of the patient or the etiology of cirrhosis.

In keeping with the significant reductions in viable fecal counts of both aerobic and anaerobic Gram-negative bacteria, treatment with the synbiotic and fermentable fiber preparations was associated with a significant reduction in circulating levels of endotoxin, a component of the cell walls of Gram-negative bacteria. Mean serum endotoxin levels in these groups fell by 24% and 39%, respectively. Endotoxin-induced activation of macrophages plays a key role in the pathogenesis of tumor necrosis factor-alpha overproduction and associated liver injury in animal models of alcohol-related liver disease.[29,30] However, a recent analysis of the peripheral blood mononuclear cell expression of toll-like receptor 4, responsible for signal transduction leading to the production of tumor necrosis factor-alpha in response to endotoxin, has cast doubt as to the importance of endotoxin as a stimulus for tumor necrosis factor-alpha overproduction in cirrhosis in the clinical setting, irrespective of the etiology of cirrhosis.[24]

Whether related to reduction in endotoxemia or other mechanism, the Child-Turcotte-Pugh class improved in nearly 50% of our initially Child-Turcotte-Pugh class B or C synbiotic-treated patients, a proportion significantly higher than that in placebo-treated counterparts (8%). The Child-Turcotte-Pugh class also improved in 29% of patients treated with fermentable fiber alone. In patients treated with either synbiotic or fermentable fiber alone, improvement in the Child-Turcotte-Pugh class occurred

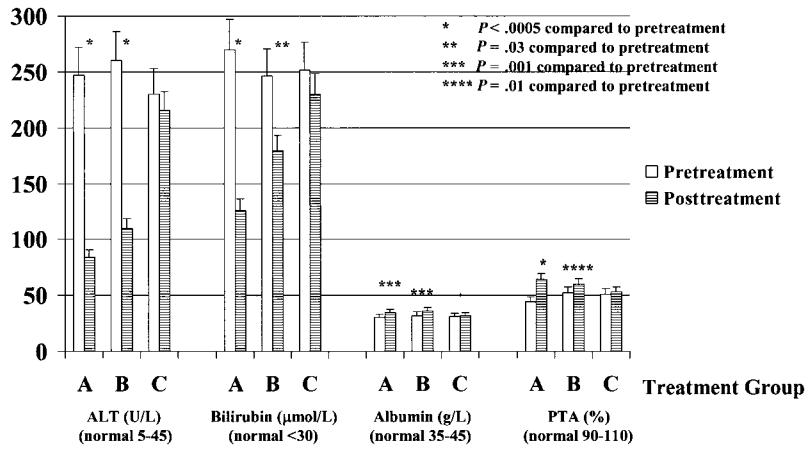

Fig. 1. Comparison of pretreatment and posttreatment serum ALT, bilirubin and albumin values, along with PTA, in Groups A, B, and C (values expressed as mean + SEM; comparisons performed using paired sample Student *t* test). ALT, alanine aminotransferase; PT, prothrombin activity.

as a result of significant improvements in the serum bilirubin and albumin levels and in prothrombin activity. These improvements occurred in association with significantly reduced hepatic necroinflammatory activity, as reflected by serial ALT levels. It is unlikely that these findings occurred as a result of resolution of some confounding acute hepatic insult, such as drug toxicity, surreptitious alcohol use, or intercurrent illness, rather than synbiotic or fermentable fiber treatment *per se*, in view not only of our careful clinical assessment and rigid exclusion criteria but also the fact that no such changes occurred in the placebo-treated group. Experience in experimental animals suggests that oral supplementation with probiotics can protect against hepatocellular damage. In particular, rats fed lactobacilli have been shown to be protected against alcohol-induced liver damage,[31] while studies of probiotic use in a murine model of nonalcoholic fatty liver disease have found reductions in the intrahepatic expression of various molecular markers of inflammation, including nuclear factor kappa-B and tumor necrosis factor-alpha.[32,33] Further studies are required to determine the precise molecular mechanisms by which treatment with synbiotics and fermentable fiber alone significantly reduced hepatocellular necroinflammation and improved hepatic function in our cohort of patients, mostly with hepatitis B virus–related cirrhosis.

Our study is the first to examine the impact of synbiotics and fermentable fiber alone on MHE and other aspects of hepatic function in patients with cirrhosis. Our demonstration of improvement in MHE in association with such treatments extends the findings of four previously published studies examining the effect of supplementation with probiotics on HE in patients with cirrhosis.[34–37] In addition to the use of probiotics (*Lactobacillus acidophilus* or *Enterococcus faecium* SF68) rather than synbiotics or fermentable fiber alone, these studies, which involved small numbers of patients, differed from ours in a number of other respects, including the recruitment of patients with overt HE rather than MHE, and the lack of a placebo-treated group. Despite the latter constraint, all 4 studies suggested possible efficacy. Notably, patients who responded to treatment with *Enterococcus faecium* SF68 maintained clinical improvement during a 2-week period off-treatment, whereas encephalopathy status typically returned to baseline during drug-free periods in lactulose-treated patients.[37] Future studies of the effect of synbiotics and fermentable fiber alone on HE, using a more extensive battery of neuropsychological tests than we employed in this current report, should similarly assess the durability of clinical response following treatment withdrawal, along with the effects of longer durations of treatment.

In contrast to our findings that strategies to reduce the colonic content of urease-positive gut flora significantly reduce blood ammonia levels and reverse MHE in the majority of cirrhotic patients with this disorder, eradication of *Helicobacter pylori*, a particular urease-positive bacterium that infects gastric mucosa, is of no benefit in either regard.[38,39] These findings suggest that urease-positive flora in the colon contribute more substantially to blood ammonia levels than does *Helicobacter pylori*, whether as a function of a greater number of urease-producing bacteria in the colon compared to *Helicobacter pylori* in the stomach, the more alkaline pH of colonic compared to gastric secretions favouring enhanced ammonia diffusion from the former site or both mechanisms.

We conclude that treatment with synbiotics or fermentable fiber is an alternative to use of non-absorbable disaccharides, such as lactulose, for the management of MHE in patients with cirrhosis. Significant reductions in viable counts of potentially pathogenic gut flora occur with both treatments. The possibility that oral supplementation with synbiotics or fermentable fiber may reduce the incidence of infective complications related to the extraintestinal translocation of pathogenic gut flora in cirrhotic patients, such as spontaneous bacterial peritonitis, warrants investigation, especially in view of the poor prognosis associated with infection in this group.

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