Increased Lipopolysaccharide Binding Protein in Cirrhotic Patients With Marked Immune and Hemodynamic Derangement

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Intestinal bacterial overgrowth and translocation, both common in cirrhosis with ascites, may lead to the activation of monocytes and lymphocytes, increased levels of proinflammatory cytokines, and enhanced synthesis of nitric oxide present in cirrhosis. Bacterial endotoxin promotes the synthesis of lipopolysaccharide (LPS)-binding protein (LBP), and forms a LPS-LBP complex that binds to CD14. This study was designed to evaluate LBP levels and their correlation to the immune response and the hemodynamic status in cirrhotic patients. Plasma LBP, endotoxin, soluble CD14 (sCD14), cytokines, renin, nitrites, and systemic vascular resistance were determined before and 4 weeks after norfloxacin or placebo in 102 cirrhotic patients and 30 controls. LBP was elevated in 42% of ascitic cirrhotic patients (15.7 ± 0.7 versus 6.06 ± 0.5 μg/mL, $P <$ .01). In 60% of high LBP patients, endotoxin was within normal range. Among ascitic patients, those with high LBP showed greater ($P <$ .05) levels of sCD14, tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), nitrites + nitrates (NOx)/creatinine, and renin, and lower vascular resistance. In the cirrhotic patients with high LBP, norfloxacin normalized ($P <$ .01) LBP (from 16.6 ± 0.5 to 5.82 ± 0.8 μ g/mL) and sCD14; reduced the level of cytokines, NOx/creatinine, and renin; and increased vascular resistance; but lacked effect in patients with normal LBP. Portal pressure was unchanged after norfloxacin in another group of 18 cirrhotic patients with high and 19 with normal LBP. In conclusion, the subset of ascitic cirrhotic patients with marked immune and hemodynamic derangement is identified by increased LBP levels. Amelioration of these abnormalities by norfloxacin suggests the involvement of enteric bacteria or their products in the triggering of the process. (HEPATOLOGY 2003;37:208-217.)

Cirrhosis with ascites is often complicated by a hyperdynamic circulatory state. It has been postulated that intestinal bacterial overgrowth, altered gut permeability, and bacterial translocation, all common in cirrhosis with ascites, may exert continuous pressure on the immune system.[1-4] This disturbance is thought to lead to activation of monocytes and lymphocytes and increased serum levels of proinflammatory cytokines such as tumor necrosis factor α (TNF-α). Exposure to bacteria and their endotoxins, directly or involving host cytokines, has been associated with increased synthesis of nitric oxide,[5] which may exacerbate the circulatory disarrangement of cirrhosis. However, the role of translocation of enteric bacteria in the immune and hemodynamic disturbances of cirrhosis is an unsettled issue[2,6,7] and is difficult to address experimentally in humans. This problem is partly attributable to the lack of a reliable marker that may identify individuals who suffer frequent passage of bacteria or their products to the circulation, because the bacteremia that follows bacterial translocation is episodic and transient in nature, and the half-life of endotoxin (lipopolysaccharide [LPS]) is short.[8,9]

Once in the circulation, endotoxin promotes the hepatic synthesis of LPS-binding protein (LBP), a plasma

Abbreviations: TNF-α, tumor necrosis factor α; LPS, lipopolysaccharide; LBP, lipopolysaccharide binding protein; SVR, systemic vascular resistance; HVPG, hepatic venous pressure gradient; NOx, nitrites + nitrates; IL-6, interleukin 6; sCD14, soluble CD14; sTNF-RI, soluble TNF-α receptor I.

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protein that enhances the binding of LPS to CD14.[10] CD14 is a component of the LPS receptor, expressed on the membrane of myeloid linage cells, that binds LPS-LBP complexes. CD14 is associated in the cell surface to a Toll-like receptor, which transduces the signal from the CD14-bound LPS to the cell nucleus. Endotoxin signaling triggers a cascade that leads to cytokine production and shedding of the extracellular domain of the CD14 receptor. Serum concentrations of CD14 are thought to reflect the amount of endotoxin and endotoxin-driven cell activation. LBP peaks in plasma 2 to 3 days after transient bacteriemia or endotoxinemia, and levels are increased up to 72 hours later.[9,10] Indeed, in several clinical settings, plasma LBP seems to reflect better the long-term exposure to bacteria and their endotoxins than endotoxin itself.[9,11,12] However, no study has yet investigated plasma LBP levels in cirrhosis. The goals of this study were to investigate (1) whether plasma LBP levels are increased in cirrhosis, and (2) if so, whether cirrhotic patients with high LBP levels exhibit more pronounced immune disturbance and hyperdynamic circulatory state.

Patients and Methods

Patients

The study was conducted in patients with cirrhosis and portal hypertension and in healthy controls. The protocol was approved by the local ethics committee and followed the principles of the Declaration of Helsinki. Written informed consent to participate in the study was obtained from each patient.

Inclusion criteria were age between 25 and 70 years, positive diagnosis of cirrhosis, and presence of esophageal varices. The diagnosis of cirrhosis was based on imaging, clinical, and analytical findings. Exclusion criteria were evidence of gastrointestinal bleeding, hepatic encephalopathy greater than grade II, bacterial infection, alcoholic hepatitis or treatment with nonabsorbable antibiotics in the preceding 6 months, active alcoholism, presence of hepatocellular carcinoma, serum creatinine $>$ 1.5 mg/dL, hyperglycemia (serum glucose $>$ 120 mg/dL), malnutrition, treatment with vasoactive drugs, history of arterial hypertension, congestive heart failure or arterial occlusive disease, and refusal to participate. Bacterial infection was ruled out by clinical history, physical examination, differential and total white blood cell count, analysis and culture of urine, thoracic radiograph, and, in ascitic patients, by culture and white blood cell count of ascitic fluid. The control population was composed of healthy subjects who were age- and sex-matched with the patients. Organic disease was excluded by clinical history, physical examination, electrocardiogram, and routine chemical analyses.

Study Protocols

Patients fulfilling all inclusion criteria and no exclusion criteria underwent 2 different protocols. Protocol 1 included 102 cirrhotic patients and 30 healthy controls. The goals of this protocol were to evaluate LBP plasma levels in cirrhosis, to test whether cirrhotic patients with increased LBP levels had more pronounced immune abnormalities and hyperdynamic circulatory state, and to assess the effect of norfloxacin on immune and systemic hemodynamic parameters. Ascites was detected by ultrasonography in 71 of these 102 patients (70%). Protocol 2 included a different set of 37 cirrhotic patients. The goal of this second protocol was to determine the effect of norfloxacin on splanchnic hemodynamics.

Study Design

One week before the study onset, selected patients were placed on a sodium-restricted diet (80 mmol/d) and diuretics were withdrawn. Patients and controls were examined in the supine position after an overnight fast. After a 1-hour period of rest, noninvasive hemodynamic measurements (arterial pressure, cardiac output by echocardiography, forearm blood flow by plethysmography) were performed, and blood was drawn in subjects included in Protocol 1. Under similar experimental conditions, patients included in Protocol 2 underwent measurements of portal pressure, hepatic blood flow, arterial pressure and cardiac output by thermodilution, and withdrawal of blood.

Once these measurements were made, the patients and controls included in Protocol 1 and the patients included in Protocol 2 were randomly allocated according to a computer-generated sequence to receive norfloxacin (400 mg orally twice daily) or an indistinguishable placebo for 4 weeks each. A biased randomization was chosen in a 2:1 ratio. After treatment with norfloxacin or placebo, the same measurements and determinations were repeated.

Methods

Hemodynamic Measurements. Mean arterial pressure was measured with an automatic sphygmomanometer (Dinamap; Critikon, Tampa, FL). Stroke volume was measured by 2-dimensional Doppler echocardiography using the left ventricle outflow method, with an ATL HDI 5000 equipped with a 4-2 MHz transducer.[13] Cardiac output was calculated as the product of stroke volume and heart rate. Cardiac index was obtained by dividing cardiac output by body surface area. Forearm blood flow was measured in the nondominant arm by venous occlusion plethysmography (EC 5-R; Hokanson, Issaquah, WA), as previously described.[14] Systemic vascu-

lar resistance (SVR) was calculated as the ratio of mean arterial pressure to cardiac index × 80.

In Protocol 2, measurements of portal and cardiopulmonary pressures and cardiac output were performed by introducing a 7F balloon catheter (Medi-Tech, Inc, Watertown, MA) into the main right hepatic vein and a Swan-Ganz catheter into the pulmonary artery respectively, using a multichannel recorder (Hellige, Freiburg, Germany).[15] Portal pressure was estimated in terms of the hepatic venous pressure gradient (HVPG) as the difference between the wedged and free hepatic venous pressures. Hepatic blood flow was determined during continuous infusion of indocyanine green (0.2 mg/min; ICG; Pulsion Medical Systems, Munich, Germany).

Assays. Blood samples were collected into endotoxin-free tubes (Endo Tube ET; Chromogenix AB, Sweden), centrifuged, and plasma samples stored at −80°C until analysis. Plasma LBP was measured by immunometric sandwich assay with a chemiluminescent substrate using an automated analyzing system (Immulite LBP; DPC, Los Angeles, CA). The lower limit of assay sensitivity was 0.2 μg/mL. Plasma endotoxin was measured by a quantitative, chromogenic Limulus amebocyte lysate assay (QCL-1000; BioWhittaker Inc, Walkersville, MD). Endotoxin inhibitors were removed from plasma by dilution with sterile endotoxin-free water and by treatment of the mixture at 75°C for 5 minutes. The lower limit of detection of this assay was 0.05 EU/mL. In normal plasma, endotoxin levels are typically <0.5 EU/mL. Intra-assay and interassay coefficients were 9.2% and 10.3% for endotoxin, and 3.0% and 4.1% for LBP, respectively. Serum concentrations of nitric oxide metabolites (nitrates + nitrites [NOx]) were determined by chemiluminescence (Nitric Oxide Analyzer; NOA 280, Sievers Instruments, Boulder, CO). Because the glomerular handling of NOx parallels that of creatinine, values of NOx (in nanomoles per milliliter) in serum were corrected with serum creatinine (in micromoles per liter). Enzyme-linked immunosorbent assay kits (Quantikine; R&D Systems, Minneapolis, MN) were used to measure soluble CD14 (sCD14), bioactive TNF-α (HS assay), interleukin 6 (IL-6, HS assay), and soluble TNF-receptor I (sTNF-RI, p55). The lower limits of detection of these assays were 125 pg/mL for sCD14, 0.18 pg/mL for TNF-α, 0.094 pg/mL for IL-6, and 3.0 pg/mL for sTNF-RI. All determinations were performed in duplicate, and the mean value was used.

Statistical Analysis

Results are shown as mean ± SE. Frequency data were compared using the Fisher's exact test. Comparison among groups was performed by one-way ANOVA, followed by a posthoc *t* test (Bonferroni). The paired Student's *t* test was used to analyze the effect of norfloxacin. The association between continuous variables was determined through linear regression analysis. A value of $P < .05$ was considered to denote significance. Statistical analysis was performed using the StatView program package (Abacus Concepts, Inc, Berkeley, CA).

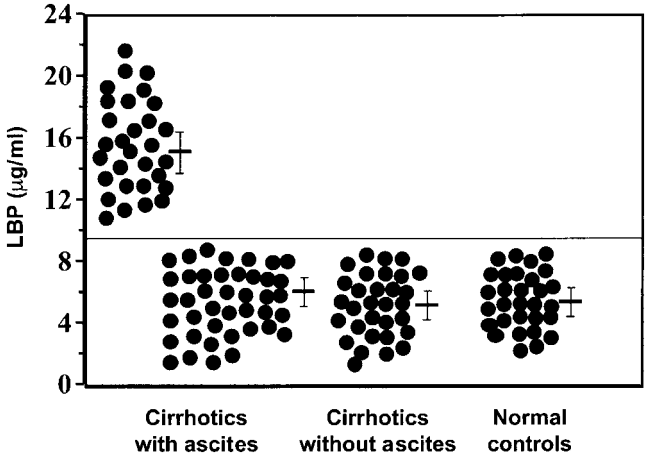

Fig. 1. Plasma LBP levels in cirrhotic patients with and without ascites and in healthy controls. Plasma LBP was significantly greater ($P < .01$) in cirrhotic patients with ascites than in the other groups. High LBP was defined as > mean + 2 SD in healthy controls (>9.62 μg/mL, **horizontal line**). Twenty-nine percent of the patients, all with ascites, showed high LBP. **Horizontal bars** and **vertical bars** represent the mean and SEM, respectively.

Results

Protocol 1: LBP Plasma Levels in Cirrhosis: Immune and Systemic Hemodynamic Abnormalities in Cirrhotic Patients With High LBP, and Their Amelioration After Intestinal Decontamination

Plasma LBP was significantly higher ($P < .01$) in cirrhotic patients with ascites (8.12 ± 0.5 μg/mL) than in cirrhotic patients without ascites (5.46 ± 0.4 μg/mL) and healthy controls (5.62 ± 0.4 μg/mL). High plasma LBP levels (defined as greater than mean ± 2 SD in healthy controls, >9.62 μg/mL) were detected in a subgroup of 30 of the 71 cirrhotic patients with ascites (42%), but in none of those without ascites ($P < .01$) (Fig. 1). We thereafter classified patients with ascites into 2 groups: patients with a high (15.7 ± 0.7 μg/mL) and those with a normal LBP concentration (6.06 ± 0.5 μg/mL).

The 2 groups of cirrhotic patients with ascites defined by LBP level showed a distinct immune and hemodynamic profile (see below), but were otherwise not clinically distinguishable (Table 1). The mean age and sex distribution in both groups was similar. Cirrhosis was secondary to alcohol abuse and to hepatitis C virus infection in approximately half of the patients of each group.

Table 1. Clinical Characteristics of Patients and Controls Included in Protocol 1

	Cirrhotic Patients With Ascites (n = 71)		Cirrhotic Patients Without Ascites (n = 31)	Healthy Controls (n = 30)
	High LBP (n = 30)	Normal LBP (n = 41)		
Age (years)	58 ± 4	56 ± 4	55 ± 3	52 ± 2
Male gender (%)	74%	70%	73%	70%
Etiology of cirrhosis (% alcohol)	53%	58%	56%	–
Child score (points)	8.8 ± 0.3*	9.0 ± 0.3*	6.1 ± 0.2	–
Grade of esophageal varices I-II/III-IV	9/21	12/29	10/21	–
Serum bilirrubin (mg/dL)	3.1 ± 0.6†‡	3.4 ± 0.7†‡	1.4 ± 0.2	0.7 ± 0.3
Serum albumin (g/L)	3.1 ± 1.7	3.2 ± 1.5	3.8 ± 0.9	4.2 ± 0.8
Prothrombin (% of normal)	46 ± 3*†	43 ± 2*†	71 ± 3†	98 ± 2
Serum creatinin (mg/dL)	1.09 ± 0.09‖	1.05 ± 0.05‖	0.84 ± 0.03	0.78 ± 0.03
Urinary sodium (mEq/d)	9 ± 6*‖	9 ± 6*‖	52 ± 5	50 ± 4

*$P < .01$ vs. cirrhotic patients without ascites.
†$P < .01$ vs. healthy controls.
‡$P < .05$ vs. cirrhotic patients without ascites.
‖$P < .05$ vs. healthy controls.

The Child score was greater in patients with ascites, but similar in ascitic patients with high and normal LBP.

Cirrhotic patients with ascites and high LBP showed significantly higher mean circulating values of endotoxin, proinflammatory cytokines (TNF-α, IL-6), and soluble receptors (sCD14 and sTNF-RI) than ascitic and nonascitic patients with normal LBP and healthy controls (Table 2). Interestingly, circulating endotoxin levels were within the normal range in most patients with high LBP (18 of 30, 60%), and in all the remaining patients. Serum concentrations of proinflammatory cytokines were significantly greater ($P < .01$) in cirrhotic patients with normal LBP (ascitic and nonascitic) than in healthy controls (Table 2). Concentrations of LBP correlated significantly with sCD14 levels ($r = 0.91$, $P < .001$), and TNF-α ($r = 0.75$, $P < .01$) in patients with ascites. These correlations were also present when patients with ascites and high LBP were separately analyzed (LBP and sCD14, $r = 0.81$, $P < .001$; LBP and TNF-α, $r = 0.64$, $P < .01$) (Fig. 2).

No significant differences were found in the mean values of LBP, endotoxin, soluble receptors, and proinflammatory cytokines between alcoholic and nonalcoholic cirrhotic patients with high LBP (LBP, 14.6 ± 0.6 vs. 15.9 ± 0.7 μg/mL; endotoxin, 0.58 ± 0.05 vs. 0.70 ± 0.07 EU/mL; sCD14, 2,476 ± 99 vs. 2,988 ± 105 ng/mL; TNF-α, 8.05 ± 0.4 vs. 9.01 ± 0.6 pg/mL; IL-6, 28.4 ± 1.2 vs. 32.8 ± 1.7 pg/mL). Neither were significant differences in the mean values of these parameters between alcoholic and nonalcoholic cirrhotic patients with normal LBP (ascitic or nonascitic).

Cirrhotic patients with ascites and high LBP showed a more pronounced hyperdynamic circulatory state and greater activation of endogenous vasoactive systems (Table 3). These patients showed lower mean arterial pressure, greater forearm blood flow and cardiac index, and lower SVR than did patients with ascites and normal LBP. The degree of activation of endogenous vasoactive systems (plasma renin activity and aldosterone concentra-

Table 2. Plasma Concentrations of Endotoxin and of Inflammatory Markers in Patients and Controls Included in Protocol 1

	Cirrhotic Patients With Ascites (n = 71)		Cirrhotic Patients Without Ascites (n = 31)	Healthy Controls (n = 30)
	High LBP (n = 30)	Normal LBP (n = 41)		
Endotoxin (EU/mL)	0.68 ± 0.06*†‡	0.37 ± 0.03	0.34 ± 0.03	0.29 ± 0.04
sCD14 (ng/mL)	2,676 ± 104*§‖	1,552 ± 98	1,498 ± 132	1,384 ± 138
TNF-α (pg/mL)	8.50 ± 0.5†‡¶	5.34 ± 0.4‡§	3.81 ± 0.3	1.74 ± 0.4
Interleukin-6 (pg/mL)	31.6 ± 1.6†‡¶	16.3 ± 1.5‡§	11.2 ± 0.9	3.1 ± 0.5
sTNF-receptor I (pg/mL)	2,442 ± 354†‡¶	1,510 ± 88	1,158 ± 68	818 ± 56

*$P < .05$ vs. cirrhotics with ascites and normal LBP.
†$P < .01$ vs. cirrhotics without ascites.
‡$P < .01$ vs. healthy controls.
§$P < .05$ vs. cirrhotics without ascites.
‖$P < .05$ vs. healthy controls.
¶$P < .01$ vs. cirrhotics with ascites and normal LBP.

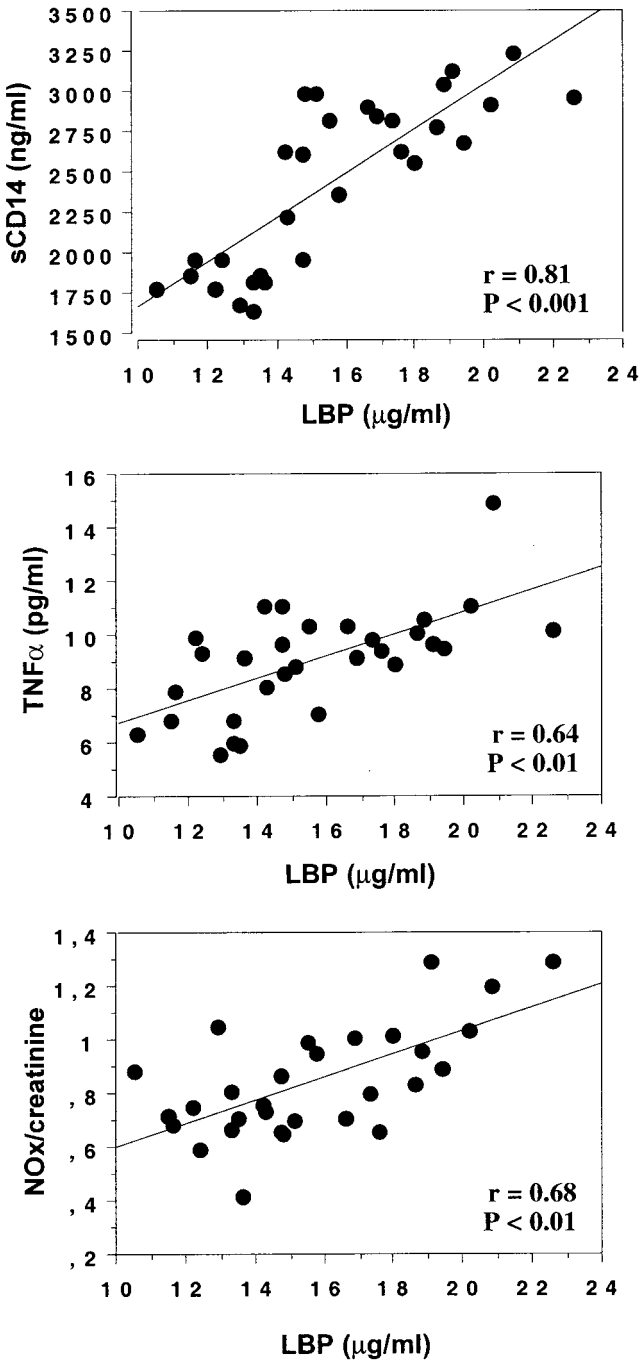

Fig. 2. Correlation of LBP plasma levels with sCD14, TNF-α, and NOx/creatinine ratio in cirrhotic patients with ascites and high LBP.

tion) and the NOx/creatinine ratio were also enhanced in ascitic cirrhotic patients with high LBP compared with the remaining subjects. In addition, these variables were also higher ($P < .05$) in cirrhotic patients with ascites and normal LBP than in controls. Among cirrhotic patients with ascites, the NOx/creatinine ratio correlated significantly with LBP ($r = 0.78$, $P < .001$) and TNF-α ($r = 0.71$, $P < .001$) levels, as well as with SVR values ($r = -0.72$, $P < .01$). The NOx/creatinine ratio also correlated with LBP ($r = 0.68$, $P < .01$) and with TNF-α ($r = 0.62$, $P < .01$) when patients with ascites and high LBP were separately analyzed (Fig. 2). Concentrations of LBP also correlated significantly with SVR ($r = -0.41$, $P < .01$) and with serum creatinine values ($r = 0.32$, $P < .05$) in patients with ascites.

Norfloxacin normalized LBP in patients with elevated basal levels, from 16.6 ± 0.5 to 5.82 ± 0.8 μg/mL ($P < .001$), a final value not significantly different than that recorded in nonascitic cirrhotic patients and healthy controls (Fig. 3). Norfloxacin also normalized the level of sCD14, and significantly reduced concentrations of proinflammatory cytokines (TNF-α and IL-6) (Table 4). In these ascitic cirrhotic patients with high LBP, norfloxacin led to a nonsignificant increase in mean arterial pressure ($P = .07$) and a significant decrease in cardiac index ($P < .01$) (Table 4). As a result of these hemodynamic changes, SVR increased by 15 ± 4%, from 1,582 ± 144 to a final value of 1,884 ± 124 dyn · sec · cm^{-5} ($P < .001$); not significantly different from that found in ascitic cirrhotic patients with normal basal LBP levels (Fig. 3). NOx/creatinine ratio, plasma renin activity, and aldosterone concentration were significantly reduced by norfloxacin in ascitic cirrhotic patients with high LBP. These variables were similar to those recorded in cirrhotic patients with ascites but normal LBP. No significant changes in biochemical, immune, or hemodynamic variables were observed in cirrhotic patients with high LBP receiving a placebo (Table 4).

In cirrhotic patients (ascitic and nonascitic) with normal LBP and in healthy controls, neither norfloxacin nor placebo led to significant changes in LBP levels, proinflammatory cytokines, endogenous vasoactive systems, or systemic hemodynamics (Table 5).

Protocol 2: Norfloxacin Fails to Modify Splanchnic Hemodynamics in Cirrhotic Patients With High LBP

LBP was increased in 18 of the 37 (48%) cirrhotic patients with ascites included in this protocol. Patients showing high and normal LBP baseline values were similar in terms of etiology of cirrhosis (percent of alcohol, 54% vs. 52%), Child score (9.2 versus 9.4 points), and serum creatinine (0.89 vs. 0.91 mg/dL). Patients with high LBP showed greater values of sCD14 (2,855 ± 146 vs. 1,645 ± 101 ng/mL, $P < .001$), TNF-α (8.12 ± 0.5 vs. 4.9 ± 0.4 pg/mL, $P < .001$), and IL-6 (34.6 ± 1.7 vs. 12.2 ± 1.3 pg/mL), greater cardiac index (measured by thermodilution) (5.14 ± 0.3 vs. 4.26 ± 0.3 mL/min · m^2, $P < .05$), and lower mean arterial pressure (76 ± 2 vs. 86 ± 2 mm Hg, $P < .01$) and SVR (1,156 ± 129 versus 1,615 ± 121 dyn · sec · cm^{-5}, $P < .01$) than those with

Table 3. Systemic Hemodynamics and Endogenous Vasoactive Systems in Patients and Controls Included in Protocol 1

	Cirrhotic Patients With Ascites (n = 71)		Cirrhotic Patients Without Ascites (n = 31)	Healthy Controls (n = 30)
	High LBP (n = 30)	Normal LBP (n = 41)		
Mean arterial pressure (mm Hg)	81 ± 3*†‡	85 ± 3§	88 ± 3§	99 ± 2
Cardiac index (mL/min · m^2)	4.10 ± 0.2*†‡	3.48 ± 0.2‡	3.06 ± 0.2§	2.44 ± 0.1
Systemic vascular resistance (dyn · s · cm^{-5})	1,612 ± 142*†‡	2,056 ± 147‡	2,392 ± 132§	3,249 ± 94
Forearm blood flow (mL/min%)	4.8 ± 0.3*†‡	4.1 ± 0.2‡	3.5 ± 0.2§	2.1 ± 0.08
NOx/creatinine ratio	0.88 ± 0.1†‡‖	0.51 ± 0.05§	0.38 ± 0.04	0.22 ± 0.03
Plasma renin activity (ng/mL · h)	8.6 ± 0.9†‡‖	5.0 ± 0.7§	1.2 ± 0.1	0.9 ± 0.03
Plasma aldosterone concentration (ng/dL)	88.2 ± 11†‡‖	45.4 ± 8‡¶	17.9 ± 4	6.2 ± 1

*$P < .05$ vs. cirrhotic patients with ascites and normal LBP.
†$P < .01$ vs. cirrhotic patients without ascites.
‡$P < .01$ vs. healthy controls.
§$P < .05$ vs. healthy controls.
‖$P < .01$ vs. cirrhotic patients with ascites and normal LBP.
¶$P < .05$ vs. cirrhotic patients without ascites.

normal LBP. HVPG (22.5 ± 2.2 vs. 21.8 ± 2.1 mm Hg, NS) and hepatic blood flow (1,402 ± 156 vs. 1,395 ± 145 mL/min, NS) were similar in both groups of subjects.

As in Protocol 1, norfloxacin significantly lowered LBP in patients with elevated levels of this protein, but had no effect on HVPG and hepatic blood flow (Table 6). Neither were these variables significantly affected by the placebo in patients with high LBP. Splanchnic hemodynamic variables remained unchanged in ascitic cirrhotic patients with normal LBP receiving norfloxacin or placebo (data not shown).

Discussion

In this prospective study, we show that plasma LBP levels identify 2 distinct groups of subjects with cirrhosis. High LBP plasma levels were detected in one third of cirrhotic patients, all with ascites. Cirrhotic patients with high LBP showed greater circulating levels of the proinflammatory cytokines, TNF-α and IL-6, and the soluble receptors, sCD14 and sTNF-RI. They also showed enhanced activation of endogenous vasoactive systems and more intense features of a hyperdynamic circulatory state. Treatment with norfloxacin normalized LBP and sCD14 levels and improved the proinflammatory immune status and the hyperdynamic circulatory state.

Interestingly, endotoxin was only detected in one third of patients with high LBP, and in no patient with normal LBP. The considerable number of patients with high LBP and undetectable endotoxin levels could be accounted for by the fact that bacteremia is intermittent and episodic in nature and the half-life of endotoxin is short (10 to 30 minutes), whereas the half-life of LBP is substantially longer (8 to 11 minutes). These drawbacks are reflected in the rate of endotoxin detection in cirrhosis, which ranges from 0 to 93% in several studies.[6,16-20] In the present study, elevated LBP levels identified a subset of ascitic cirrhotic patients with an exacerbated proinflammatory immune response and a more marked deranged hemody-

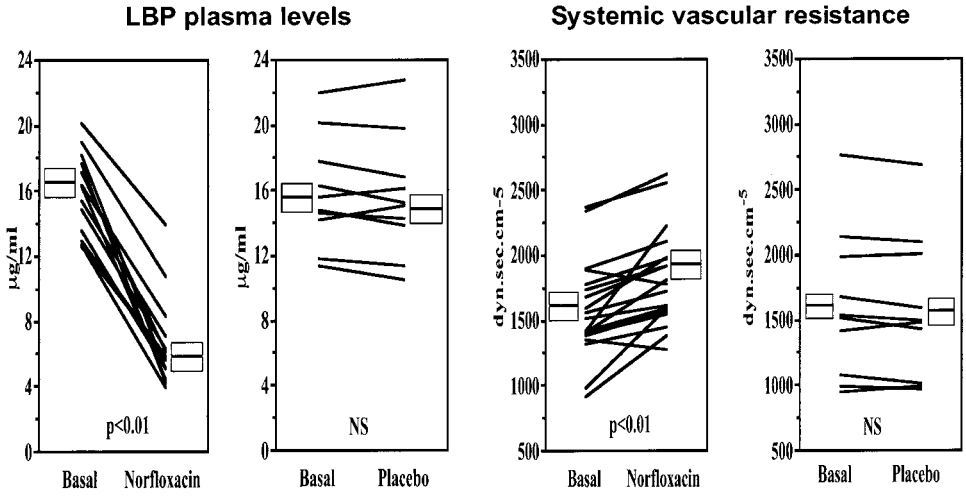

Fig. 3. Effect of norfloxacin or placebo on individual plasma LBP levels and systemic vascular resistance values in patients with high LBP. Norfloxacin, but not placebo, significantly reduced LBP ($P < .01$) in patients with high LBP. **Horizontal bars** and **squares** represent the mean and SEM, respectively.

Table 4. Plasma Concentrations of LBP, Inflammatory Markers, Systemic Hemodynamics and Endogenous Vasoactive Systems in Ascitic Cirrhotic Patients With High LBP at Baseline and After 4-Week Norfloxacin (n = 20) or Placebo (n = 10) Administration (Protocol 1)

	Baseline	Norfloxacin	Baseline	Placebo
LBP (μg/mL)	16.6 ± 0.5	5.82 ± 0.8*†	15.0 ± 0.7	14.4 ± 0.6
Endotoxin (EU/mL)	0.68 ± 0.06	0.32 ± 0.03*†	0.62 ± 0.04	0.66 ± 0.05
Soluble CD14 (ng/mL)	2,445 ± 122	1,394 ± 120*†	2,628 ± 138	2,660 ± 138
TNF-α (pg/mL)	8.48 ± 0.5	4.63 ± 0.6*†	8.49 ± 0.5	8.63 ± 0.5
Interleukin-6 (pg/mL)	32.8 ± 1.8	17.0 ± 2.5*†	30.1 ± 1.5	31.3 ± 2.0
Soluble TNF-receptor I (pg/mL)	2,650 ± 125	1,553 ± 77*†	2,496 ± 78	2,532 ± 96
Mean arterial pressure (mm Hg)	78 ± 3	83 ± 2	80 ± 3	81 ± 2
Cardiac index (mL/min · m^2)	4.22 ± 0.3	3.61 ± 0.2*‡	3.99 ± 0.2	4.10 ± 0.2
Systemic vascular resistance (dyn · sec · cm^{-5})	1,582 ± 144	1,884 ± 124*‡	1,662 ± 146	1,541 ± 122
Forearm blood flow (mL/min%)	5.2 ± 0.3	4.1 ± 0.3*‡	4.9 ± 0.3	5.0 ± 0.1
NOx/creatinine ratio	0.84 ± 0.1	0.38 ± 0.07*‡	0.81 ± 0.06	0.78 ± 0.05
Plasma renin activity (ng/mL · h)	8.4 ± 0.6	4.4 ± 0.8*†	8.6 ± 0.7	8.5 ± 0.6
Plasma aldosterone concentration (ng/dL)	88.4 ± 14	42.6 ± 10*	87.4 ± 12	84.6 ± 11

*$P < .01$ norfloxacin vs. baseline.
†$P < .01$ norfloxacin vs. placebo.
‡$P < .05$ norfloxacin vs. placebo.

namic status that could not be identified by elevated endotoxin levels. To avoid possible confounding factors, patients with recent complications of cirrhosis, recent bacterial infection, or current or past pathologic conditions with possible effects on the immune system were excluded. Patients undergoing active alcohol ingestion were also excluded because alcoholism has been associated with increased intestinal permeability and endotoxinemia.[20,21]

Endotoxin or endotoxin-containing particles (including intact gram-negative bacteria) form complexes with LBP and activate monocytes and tissue macrophages. This leads to the production of proinflammatory cytokines, release of sCD14, and further production of LBP.[10,12,22] Thus, the high correlation found between serum concentrations of LBP and sCD14 is not surprising. Previous evidence indicates that increased LBP and sCD14 plasma concentrations might be related to passage of bacteria or their products (endotoxins) to the circulation.[10,12,23] Indeed, increased concentrations of LBP and sCD14 receptors have been shown to reflect the amount of endotoxin/monocyte interaction that takes place over the long term in several clinical situations.[8-12,24]

A link has been proposed among bacterial translocation, cytokine production, nitric oxide synthesis, and hemodynamic abnormalities in cirrhosis.[2,7,17,25] Bacteria or bacteria-delivered endotoxin promotes the release of proinflammatory cytokines, in particular TNF-α, which is a widely known stimulator of inducible and endothelial nitric oxide synthase activity.[26,27] In agreement with this, cirrhotic patients with high LBP showed an inflammatory immune response that was characterized by greater levels

Table 5. Plasma Concentrations of LBP, Inflammatory Markers, Systemic Hemodynamics, and Endogenous Vasoactive Systems in Cirrhotic Patients (Ascitic and Nonascitic) With Normal LBP at Baseline and After 4-Week Norfloxacin (n = 45) or Placebo (n = 23) Administration (Protocol 1)

	Baseline	Norfloxacin	Baseline	Placebo
LBP (μg/mL)	5.61 ± 0.6	5.24 ± 0.7	5.77 ± 0.5	5.82 ± 0.6
Endotoxin (EU/mL)	0.35 ± 0.02	0.32 ± 0.03	0.34 ± 0.03	0.33 ± 0.04
Soluble CD14 (ng/mL)	1,514 ± 101	1,510 ± 96	1,530 ± 98	1,541 ± 128
TNF-α (pg/mL)	4.42 ± 0.4	4.63 ± 0.5	4.91 ± 0.5	4.86 ± 0.6
Interleukin-6 (pg/mL)	16.2 ± 1.2	17.0 ± 1.5	15.7 ± 0.7	14.9 ± 1.8
Soluble TNF-receptor I (pg/mL)	1,266 ± 91	1,316 ± 84	1,423 ± 72	1,398 ± 86
Mean arterial pressure (mm Hg)	87 ± 2	85 ± 3	85 ± 3	87 ± 2
Cardiac index (ml/min · m^2)	3.32 ± 0.2	3.30 ± 0.3	3.44 ± 0.2	3.68 ± 0.2
Systemic vascular resistance (dyn · sec · cm^{-5})	2,092 ± 128	2,118 ± 134	1,979 ± 119	1,894 ± 112
Forearm blood flow (mL/min%)	3.9 ± 0.2	3.6 ± 0.3	3.6 ± 0.2	3.7 ± 0.3
NOx/creatinine ratio	0.42 ± 0.06	0.44 ± 0.1	0.46 ± 0.07	0.43 ± 0.1
Plasma renin activity (ng/mL · h)	3.1 ± 0.2	3.6 ± 0.3	3.7 ± 0.7	3.3 ± 0.4
Plasma aldosterone concentration (ng/dL)	31.7 ± 9	35.8 ± 10	33.1 ± 12	34.5 ± 10

NOTE. Three patients of the 48 randomized to receive norfloxacin and 1 of the 24 to receive placebo were lost for follow-up.

Table 6. Splanchnic Hemodynamics, Cardiopulmonary Pressures, and Systemic Vascular Resistance in Ascitic Cirrhotic Patients With High Basal Level of LBP at Baseline and After 4-Week Norfloxacin (n = 12) or Placebo (n = 6) Administration (Protocol 2)

	Baseline	Norfloxacin	Baseline	Placebo
LBP (μg/mL)	16.1 ± 0.6	6.09 ± 0.4*†	15.8 ± 0.4	14.9 ± 0.5
Soluble CD14 (ng/mL)	2,972 ± 128	1,388 ± 126*†	2,766 ± 141	2,720 ± 134
Mean arterial pressure (mm Hg)	75 ± 2	87 ± 3‡§	77 ± 3	76 ± 2
Cardiac index (mL/min · m^2)	5.16 ± 0.3	4.50 ± 0.3‡	5.14 ± 0.2	5.22 ± 0.4
Systemic vascular resistance (dyn · sec · cm^{-5})	1,182 ± 126	1,459 ± 112‡§	1,146 ± 142	1,167 ± 102
Right atrial pressure (mm Hg)	4.02 ± 0.8	4.0 ± 1	4.3 ± 1	4.2 ± 1
Pulmonary capillary pressure (mm Hg)	7.8 ± 1	7.4 ± 1	7.6 ± 1	7.7 ± 1
Wedged hepatic venous pressure (mm Hg)	29.8 ± 2.2	28.6 ± 2.4	27.9 ± 2.4	27.2 ± 2.1
Free hepatic venous pressure (mm Hg)	6.9 ± 0.6	6.0 ± 0.8	6.1 ± 0.8	6.3 ± 0.7
Hepatic venous pressure gradient (mm Hg)	22.8 ± 2.4	22.0 ± 2.3	21.2 ± 2.2	20.7 ± 2.0
Hepatic blood flow (mL/min)	1,358 ± 152	1,382 ± 122	1,428 ± 148	1,399 ± 142

*$P < .01$ norfloxacin vs. baseline.
†$P < .01$ norfloxacin vs. placebo.
‡$P < .05$ norfloxacin vs. baseline.
§$P < .05$ norfloxacin vs. placebo.

of TNF-α than that of the remaining groups. Elevated TNF-α levels correlated both with LBP and sCD14 concentrations in cirrhotic patients with high LBP. In addition, cirrhotic patients with high LBP showed enhanced peripheral vasodilation, greater activation of endogenous vasoactive systems, and enhanced NOx levels (an index of nitric oxide production). Lower SVR in ascitic cirrhotic patients with high LBP, compared with those with normal LBP, was the consequence of lower mean arterial pressure and a greater cardiac index and peripheral blood flow. In this study, cardiac output was measured in Protocols 1 and 2 by two different methods, echocardiography and thermodilution, respectively. Although the correlation between both methods is good, echocardiography underestimates the value of cardiac output measured by thermodilution, the latter considered as the clinical standard.[28] This fact explains the greater values of SVR observed in Protocol 1 than in Protocol 2 patients. The profound hemodynamic disturbance of cirrhotic patients with high LBP may in part be caused by the increased amounts of proinflammatory cytokines with a concomitant excess synthesis of nitric oxide. This point is reflected by the significant correlations observed between the serum concentrations of LBP and NOx and those of NOx and TNF-α.

To analyze the possible contribution of enteric bacteria and their products to the increased LBP levels and the immune and hemodynamic abnormalities observed in cirrhosis, we tried to reduce the enteric bacterial load with norfloxacin. Several studies in patients and experimental models of cirrhosis have suggested that decontamination of the digestive tract with antibiotics acting against gram-negative aerobic bacilli reduces bacterial overgrowth and translocation, endotoxemia, and the resultant endothelial activation.[17,29-32] In the present study, norfloxacin normalized LBP and sCD14 levels and ameliorated proinflammatory cytokine activation, nitric oxide production, and peripheral vasodilation in ascitic cirrhotic patients with high LBP. The potential involvement of enteric bacterial products in increased nitric oxide synthesis in cirrhosis seems to be limited to a specific subset of patients with ascites, those with high LBP. Indeed, this fluoroquinolone did not modify NOx levels in patients with normal LBP, whether ascitic or nonascitic. The lack of effect of norfloxacin on the immune and systemic hemodynamic variables of patients with normal LBP does not support the possible direct immunomodulatory or vasoactive activity of the drug. Decontamination of the bowel lowered, but did not normalize, circulating proinflammatory cytokines in patients with high LBP, suggesting that bacteria-induced immune system stimulation is not the sole contributor to enhanced endogenous cytokine production in cirrhosis. Impaired hepatic clearance and immune activation secondary to tissue injury could be further contributing factors.[21,33] Contribution of bacteria to nitric oxide overproduction in decompensated cirrhosis agrees with the greater vasoconstrictor response to nitric oxide inhibitors observed in these patients as compared with those with compensated cirrhosis.[34]

During experimental endotoxemia, upregulation of hepatic endothelin-1 and increased synthesis of endothelin-1 and cyclooxygenase products act in combination to increase portal venous resistance.[35] Accordingly, in Protocol 2 we tested whether norfloxacin might reduce HVPG in cirrhotic patients with ascites and high LBP. Whereas norfloxacin diminishes systemic hyperemia and activation of endogenous vasoactive systems, it fails to modify HVPG and hepatic blood flow. Remarkably, nor-

floxacin reduces the production of nitric oxide, a molecule with prominent relaxing effects on hepatic sinusoidal vessels. It is thought that the effect of the quinolone on the enteric bacterial load causes both a decrease in flow and an increase in hepatic vascular resistance, which could account for the lack of effect on portal pressure.

Taken together, our results support the hypothesis that repeated episodic passage of enteric bacterial products to the circulation contributes to the immune and hemodynamic derangement of the cirrhotic patient with ascites. It is proposed that endotoxinemia may act on the endotoxin receptor to promote increased LBP and sCD14 levels, on the one hand, and excess proinflammatory cytokine production, on the other. The latter would contribute to further deteriorating the hemodynamic status of cirrhosis by enhancing nitric oxide production. In keeping with these findings, bacterial translocation has only been shown in a subset of cirrhotic rats with ascites.[2] More profound peripheral vasodilation and greater TNF-α–mediated endothelial nitric oxide production were shown in these animals than in those not suffering bacterial translocation. Normalization of LBP and sCD14 plasma levels by norfloxacin points to the gut as the source of bacterial products promoting their synthesis. The recent finding that norfloxacin reverts enhanced endothelium nitric oxide–mediated vasodilation in patients with cirrhosis is also in line with the present results.[7]

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