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Inhibition of Nitric Oxide Production and the Effects of Arginine and *Lactobacillus* Administration in an Acute Liver Injury Model

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Objective

To study the effect of inhibiting nitric oxide production and the effects of arginine and lactobacilli administration in an acute liver injury (LI) model.

Summary Background Data

Infectious complications caused by enteric bacteria are common in patients with liver diseases and those who have undergone liver surgery. Increased bacterial translocation has been proposed as one underlying mechanism. Lactobacilli constitute an integral part of the normal gastrointestinal microecology; they are involved in host metabolism and have many beneficial properties. Arginine has numerous roles in cellular metabolism and may be metabolized by lactobacilli in some cases. We have previously shown that rectal administration of *Lactobacillus plantarum* DSM 9843 (strain 299v), with and without arginine, in an acute LI model significantly reduces the extent of the LI and reduces bacterial translocation. To clarify the pathogenetic mechanisms, we studied the role of nitric oxide in the effects of *L. plantarum* and arginine in acute LI, as determined by bacterial translocation, ileal, cecal, and colonic nucleotides, RNA, and DNA.

Methods

Male Sprague-Dawley rats were used. *L. plantarum*, 2% arginine, and/or N-nitro-L-arginine methyl ester (L-NAME), as appropriate, were administered rectally once daily for 8 days. Acute LI was induced on the eighth day by intraperitoneal injection of D-galactosamine (1.1 g/kg body weight), and samples were collected after 24 hours. Bacterial translocation was evaluated by culture of portal and arterial blood, mesenteric lymph nodes, and liver tissue. Liver enzymes and bilirubin were assayed in the serum. The bacterial load in the cecum and colon was determined. Ileal, cecal, and colonic mucosal nucleotides, RNA, and DNA were evaluated.

Results

The levels of liver enzymes and bilirubin were lower in liver-injured rats supplemented with arginine and *Lactobacillus*, and this effect was abolished by the addition of L-NAME. Inhibition of nitric oxide production (by L-NAME) increased bacterial translocation in many groups. L-NAME administration increased the cecal and colonic bacterial count and decreased the levels of mucosal nucleotides, RNA, and DNA.

Conclusions

Inhibition of nitric oxide production modulated the effects of arginine and *L. plantarum* in this acute LI model. L-NAME potentiated the LI, as indicated by elevation of liver enzymes and bilirubin, and it also increased bacterial translocation and the cecal and colonic bacterial count. Increased bacterial translocation could be one of the mechanisms by which LI is potentiated.

Arginine has numerous roles in cellular metabolism.[1] It has a potent effect on the body's immune system,[1] improving the immune response[2] and stimulating lymphocyte activity.[3] Arginine is the only physiologic substrate for the production of nitric oxide through the action of nitric oxide synthase (NOS).[4] Nitric oxide is a potent effector molecule in numerous biochemical pathways associated with sepsis in humans and in other mammals. Its functions include cytotoxicity for bacteria, fungi, and parasites; support of chemotaxis; inhibition of platelet aggregation and thrombosis; and free radical detoxification.[5]

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Lactobacilli constitute an integral part of the normal gastrointestinal microecology and are involved in host metabolism.[6] They possess antimicrobial activity[6–9] and can inhibit the growth of various potentially pathogenic bacteria.[6,9,10] They stimulate host immunity,[11] enhance host resistance against infection by activating liver and peritoneal macrophages,[12] and enhance the intestinal immune function.[13] They are also an important growth stimulus for the intestinal mucosa.[14] In some *Lactobacillus* species, arginine catabolism can occur by the arginine deiminase pathway,[15] and there are reports of arginine dihydrolase activity in lactic acid bacteria.[16] It is therefore possible that the effects of lactobacilli are mediated by nitric oxide.

Bacterial infection and sepsis are serious and often fatal complications of acute liver failure, and gram-negative enteric bacteria are often the causative organisms.[17,18] It has been shown that under various adverse clinical conditions, bacterial translocation from the gut to extraintestinal sites occurs, and that this may be responsible for some of the infectious complications observed.[19] We have previously shown that rectal administration of different *Lactobacillus* strains, with and without arginine, in an acute liver injury (LI) model significantly reduces the extent of the liver failure and reduces bacterial translocation. *Lactobacillus plantarum* DSM 9843 (strain 299v) seems superior to the other *Lactobacillus* strains.[20] The mechanisms of these effects are unknown.

This experiment was designed to evaluate the role of nitric oxide in the effects of rectal administration of arginine, *L. plantarum*, and a combination of both in an acute LI model by inhibition of nitric oxide production. The effects of arginine and *L. plantarum* on bacterial translocation, ileal, cecal, and colonic mucosal nucleotides, and RNA and DNA content were also studied.

MATERIALS AND METHODS

Bacterial Strain

Lactobacillus plantarum DSM 9843 (strain 299v) was isolated from human jejunum and rectum.[21] The bacterial strains were obtained from the Laboratory of Food Hygiene, Dept. of Food Technology, Lund University, Lund, Sweden. The daily administration of the *L. plantarum* strain was about 3×10^9 colony-forming units per animal.

Experimental Design

Male Sprague-Dawley rats (BK Universal AB, Sollentuna, Sweden), weighing 200 to 300 g were used. The animals were divided into nine groups of six rats each:

- Normal
- Control acute LI
- LI + supplemented N-nitro-L-arginine methyl ester (L-NAME)
- LI + supplemented arginine
- LI + supplemented arginine + L-NAME
- LI + supplemented *L. plantarum* DSM 9843 + arginine
- LI + supplemented *L. plantarum* DSM 9843 + arginine + L-NAME
- LI + supplemented *L. plantarum* DSM 9843
- LI + supplemented *L. plantarum* DSM 9843 + L-NAME.

All animals received normal rat food (R3, Lactamin AB, Stockholm, Sweden) and water *ad libitum* throughout the experiment and were kept in a 12-hour light/dark cycle at room temperature (22°C). The experimental protocol was approved by the Animal Ethics Committee of Lund University and adhered to "Guiding Principles in the Care and Use of Animals."

Acute LI was induced on the eighth day by intraperitoneal injection of D-galactosamine (Sigma, St. Louis, MO) at a dose of 1.1 g/kg body weight.[22] The *L. plantarum*, 2% arginine (0.32 g), and L-NAME (100 mg/kg body weight) (Sigma), as appropriate, were administered rectally once daily for 8 days through a rectal tube. In the acute LI control group, normal saline was administered daily through a rectal tube for 8 days and LI was induced on the eighth day. Samples were collected 24 hours after induction of LI. Under ether anesthesia, a laparotomy was performed through a midline incision using aseptic technique. Aortic blood was collected for bacteriologic and liver enzyme tests. Samples for bacteriologic analysis were also taken from the portal blood, the caudate lobe of the liver, the mesenteric lymph nodes (samples were taken from the same area and part of the mesenteric lymph nodes in each animal), and the cecal and colonic contents.

Bacteriologic Analysis

Blood samples were immediately placed in sterile tubes containing EDTA. Tissue samples were placed in 5 ml of sterile transport medium.[21] Samples were placed in an ultrasonic bath (Millipore, Sweden) for 5 minutes and then rotated on a Chiltern (Terma-Glas, Gothenberg, Sweden) for 2 minutes. A total aerobic plate count was made by placing 1 ml of the sample on brain heart infusion agar (BHI; Difco, Detroit, MI) and incubating at 37°C for 3 days. A total anaerobic plate count was made by placing the samples on BHI and incubating under anaerobic conditions (Gas Pack System, Becton Dickinson Microbiology Systems, Cockeysville, MD) at 37°C for 3 days. After 3 days the number of colonies formed on each plate was counted, with adjustments made for the weight of the original tissue. Results for tissue samples are expressed per gram of tissue; results for blood samples are expressed per milliliter of blood.

Table 1. LIVER FUNCTION TESTS IN THE EXPERIMENTAL GROUPS

Groups	ALP (μKat/L)	Bilirubin (μmol/L)	ASAT (μKat/L)	ALAT (μKat/L)
Normal Control	7.0 ± 0.7	4.6 ± 0.7	6.0 ± 1.5	5.0 ± 0.9
Liver injury (LI)	16.7 ± 1.3	17.2 ± 3.3	81.0 ± 11.7	87.8 ± 7.6
LI + L-NAME	18.7 ± 0.8	17.7 ± 3.4	116.2 ± 9.6*	94.2 ± 6.0
LI + Arginine	15.1 ± 1.0	15.0 ± 2.1	61.5 ± 4.4*†‡§‖	52.2 ± 3.0*†‡§
LI + Arginine + L-NAME	17.5 ± 1.7	20.8 ± 3.2	90.2 ± 10.1	82.0 ± 16.1
LI + Arginine + Lb. plantarum	13.2 ± 0.7†	7.2 ± 0.9‡‖	24.7 ± 2.6*†‡‖	26.8 ± 2.7*†‡‖
LI + Arginine + Lb. plantarum + L-NAME	18.3 ± 0.8	12.5 ± 2.6	46.3 ± 3.3*†‡‖	33.2 ± 3.5*†‡‖
LI + Lb. plantarum	14.0 ± 1.5	11.2 ± 2.3	46.8 ± 5.2*†‡‖	46.3 ± 4.5*†‡‖
LI + Lb. Plantarum + L-NAME	15.4 ± 1.3	19.8 ± 3.2	94.0 ± 12.4	81.5 ± 9.0

* $p<0.05$ compared with Liver injury group.
† $p<0.05$ compared with LI + L-NAME group.
‡ $p<0.05$ compared with LI + Arginine + L-NAME group.
§ $p<0.05$ compared with LI + Arginine + Lb. plantarum group.
‖ $p<0.05$ compared with LI + Lb. plantarum + L-NAME group using pairwise multiple comparison Newman-Keuls method).
ALP = Alkaline phosphatase; ASAT = Aspartate aminotransferase; ALAT = Alanine aminotransferase; L-NAME = N^G-nitro-L-arginine methyl ester.

Intestinal Microflora

Samples taken from the cecum and colon were immediately placed in 5 ml of sterile transport medium, transferred to an ultrasonic bath, and rotated on Chiltern, as above. After a conventional dilution procedure, viable counts were obtained from the following:

- BHI agar that was incubated aerobically and anaerobically at 37°C for 72 hours (aerobic and anaerobic bacterial count, respectively)
- Rogosa agar (Oxoid, Hampshire, UK) that was incubated anaerobically at 37°C for 72 hours (lactobacilli counts)
- Violet red-bile-glucose agar (Oxoid) that was incubated aerobically at 37°C for 24 hours (*Enterobacteriaceae* counts)
- BHI agar containing gram-negative anaerobic supplement (Oxoid) that was incubated anaerobically at 37°C for 72 hours (gram-negative anaerobic bacterial counts).

Liver Function Tests

After centrifugation of the blood (1000 *g*, 10 minutes), serum bilirubin, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase levels in aortic blood were measured according to the recommendations of the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology.[23]

Intestinal Mucosal Nucleotide, RNA, and DNA Content

Mucosa was scraped off the samples from the distal small intestine, cecum, and colon using a glass slide, and weighed. Nucleotide and RNA content was measured according to Munro and Fleek,[24] and DNA was measured according to Scott et al.,[25] using different concentrations of cold perchloric acid. Light absorbency was measured at 260 nm in a Beckman DU-50 spectrophotometer (Beckman Scientific Instruments, Cambridge, UK), and readings were adjusted for the weight of mucosa.

Statistics

Values are presented as means ± SEM. Differences between groups were evaluated using the one-way analysis of variance (ANOVA) test, followed by the all-pairwise multiple comparison Student Newman-Keuls method.[26] The incidence of bacterial translocation was evaluated using Fisher's exact test. Probability levels < 0.05 were considered significant.

RESULTS

Liver Function Tests

Alkaline phosphatase levels were significantly lower in the LI + arginine + *L. plantarum* group than in the LI + L-NAME group, whereas the bilirubin level decreased significantly compared with the LI + arginine + L-NAME and LI + *L. plantarum* + L-NAME groups. Aspartate aminotransferase and alanine aminotransferase levels decreased significantly in the LI + arginine + *L. plantarum*, LI + arginine + *L. plantarum* + L-NAME, and LI + *L. plantarum* groups compared with the LI, LI + L-NAME, LI + arginine + L-NAME, and LI + *L. plantarum* + L-NAME groups, whereas alanine aminotransferase levels decreased significantly in the LI + arginine group compared with the same groups. Aspartate aminotransferase levels increased

significantly in the LI + L-NAME group compared with the LI group (Table 1).

Bacterial Translocation

The incidence of bacterial translocation to portal blood decreased significantly in the LI + arginine + *L. plantarum* group (2/6) compared with the LI + L-NAME (6/6) and LI + arginine + L-NAME (6/6) groups. Bacterial translocation to arterial blood decreased significantly in the LI + arginine + *L. plantarum* (1/6) and LI + *L. plantarum* (2/6) groups compared with the LI (6/6) and LI + L-NAME (6/6) groups. Bacterial translocation to the mesenteric lymph nodes decreased significantly in the LI + *L. plantarum* group (2/6) compared with the LI (6/6), LI + L-NAME (6/6), LI + arginine + L-NAME (6/6), and LI + arginine + *L. plantarum* + L-NAME (6/6) groups.

The total amount of bacteria translocated to the liver and mesenteric lymph nodes decreased significantly in the LI + arginine + *L. plantarum* and LI + *L. plantarum* groups compared with the LI, LI + L-NAME, LI + arginine + L-NAME, and LI + arginine + *L. plantarum* + L-NAME groups (Fig. 1).

Cecal Bacterial Count

The total aerobe count increased significantly in the LI + arginine group (8.5 ± 0.2 log colony-forming units/g content) compared with the LI (8 ± 0.1) and LI + *L. plantarum* + L-NAME (8 ± 0.1) groups. The total anaerobe count increased significantly in the LI + arginine (8.6 ± 0.2), LI + arginine + L-NAME (8.6 ± 0.1), and LI + arginine + *L. plantarum* + L-NAME (8.5 ± 0.0) groups compared with the LI group (8.2 ± 0.0). The gram-negative anaerobes increased significantly in the LI + arginine group (8.5 ± 0.2) compared with the LI group (8 ± 0.0). The *Enterobacteriaceae* in the LI + arginine + *L. plantarum* group (3.8 ± 0.9) decreased significantly compared with the LI + arginine + *L. plantarum* + L-NAME group (6.9 ± 0.1). The *Lactobacillus* count increased significantly in the LI + arginine (8.6 ± 0.2) and LI + arginine + L-NAME (8.4 ± 0.1) groups compared with the LI group (8.0 ± 0.1).

Colonic Bacterial Count

The total aerobe count increased significantly in the LI + arginine group compared with the LI group. The total anaerobe count and gram-negative anaerobe count increased significantly in the LI + arginine + L-NAME group compared with the LI and the LI + *L. plantarum* groups. The *Enterobacteriaceae* decreased significantly in the LI + *L. plantarum* group compared with the LI, LI + L-NAME, LI + arginine, LI + arginine + *L. plantarum* + L-NAME, and LI + *L. plantarum* + L-NAME groups. The *Lactobacillus* count increased significantly in the LI + L-NAME and the LI + arginine + L-NAME groups compared with the LI group (Table 2).

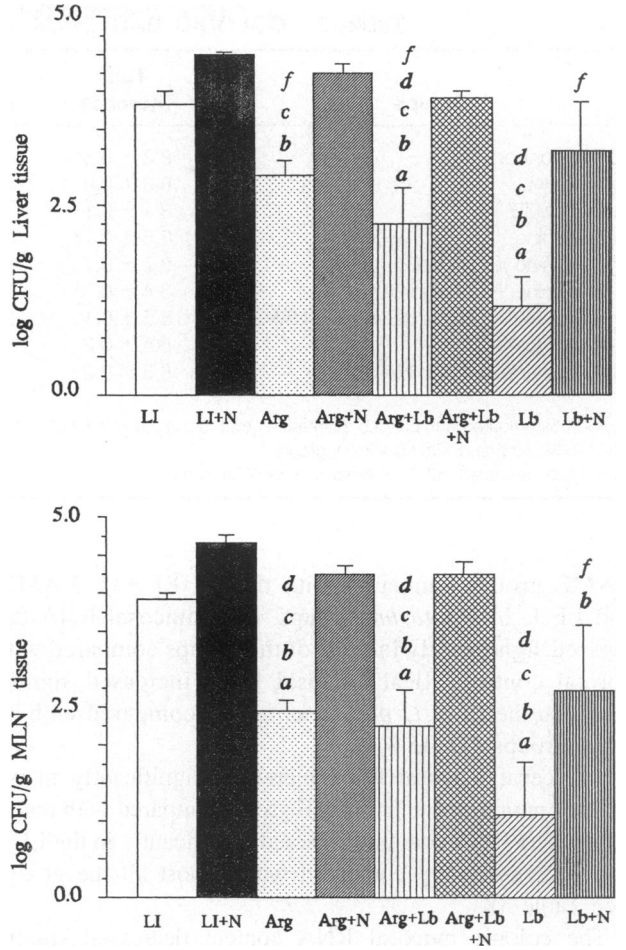

Figure 1. Total amount of bacterial translocation to the liver and mesenteric lymph nodes (log colony-forming units/g tissue). LI = liver injury; LI + N = LI + L-NAME; Arg = LI + arginine; Arg + N = LI + arginine + L-NAME; Arg + Lb = LI + arginine + *Lactobacillus plantarum*; Arg + Lb + N = LI + arginine + *L. plantarum* + L-NAME; Lb = LI + *L. plantarum*; Lb + N = LI + *L. plantarum* + L-NAME. (*a*: $p < 0.05$ *vs.* LI group; *b*: $p < 0.05$ *vs.* LI + N group; *c*: $p < 0.05$ *vs.* Arg + N group; *d*: $p < 0.05$ *vs.* Arg + Lb + N group; *f*: $p < 0.05$ *vs.* Lb group, using the all-pairwise multiple comparison Newman-Keuls method).

Intestinal Mucosal Nucleotides, RNA, and DNA Content

In the ileum, the mucosal nucleotides increased significantly in the LI, LI + L-NAME, LI + arginine, and LI + *L. plantarum* groups compared with the normal control group. The nucleotides decreased significantly after the addition of L-NAME to arginine or *L. plantarum* compared with the LI + arginine and LI + *L. plantarum* groups, respectively. Nucleotides decreased significantly in the LI + arginine + L-NAME, LI + arginine + *L. plantarum* + L-NAME, and LI + *L. plantarum* + L-

Table 2. COLONIC BACTERIAL COUNT (LOG CFU/G COLON CONTENT)

Groups	Total Aerobes	Total Anaerobes	G-ve Anaerobes	Enterobacteriaceae	Lactobacillus
Normal control	8.3 ± 0.5	8.6 ± 0.6	8.0 ± 0.2	6.2 ± 0.4	8.2 ± 0.4
Liver injury LI	8.3 ± 0.0	8.6 ± 0.0	8.4 ± 0.1	6.7 ± 0.2	8.3 ± 0.1
LI + L-NAME	8.7 ± 0.1	8.9 ± 0.1	8.8 ± 0.1	7.1 ± 0.2*	8.8 ± 0.1†
LI + Arginine	8.8 ± 0.1†	8.9 ± 0.1	8.9 ± 0.1	6.9 ± 0.3†	8.7 ± 0.1
LI + Arginine + L-NAME	8.7 ± 0.1	9.0 ± 0.1†	9.0 ± 0.1†	5.3 ± 0.1	8.9 ± 0.1†
LI + Arginine + Lb. plantarum	8.4 ± 0.1	8.6 ± 0.1	8.6 ± 0.1‡	5.5 ± 0.2	8.7 ± 0.1
LI + Arginine + Lb. plantarum + L-NAME	8.5 ± 0.1	8.7 ± 0.1	8.9 ± 0.1	7.2 ± 0.3*	8.6 ± 0.1
LI + Lb. Plantarum	8.4 ± 0.2	8.5 ± 0.2‡	8.3 ± 0.1‡	4.7 ± 1.0†	8.5 ± 0.1
LI + Lb. plantarum + L-NAME	8.5 ± 0.2	8.7 ± 0.1	8.5 ± 0.2	6.5 ± 0.6*	8.7 ± 0.2

* $p < 0.05$ compared with LI + Lb. plantarum group using pairwise multiple comparison Newman-Keuls method.
† $p < 0.05$ compared with Liver injury group.
‡ $p < 0.05$ compared with LI + Arginine + L-NAME group.

NAME groups compared with the LI, LI + L-NAME, and LI + *L. plantarum* groups. Ileal mucosal RNA decreased significantly in most of the groups compared with normal controls. Ileal mucosal DNA increased significantly in the LI + *L. plantarum* group compared with all other groups (Table 3).

The cecal mucosal RNA increased significantly in the LI + *L. plantarum* + L-NAME group compared with the LI group. The DNA content increased significantly in the LI + *L. plantarum* group compared with almost all the groups (see Table 3).

The colonic mucosal RNA content decreased significantly in the LI + L-NAME group compared with all the other groups. The mucosal DNA content increased significantly in the LI + *L. plantarum* group compared with all the other groups (see Table 3).

DISCUSSION

The results of the present study show that the inhibition of nitric oxide production by L-NAME increases LI in this acute LI model, indicating a protective role for nitric oxide. This is in agreement with other studies on other insults, where nitric oxide is known to protect the liver, as demonstrated by a reduction in hepatocellular necrosis and the release of liver enzymes.[27,28] When we added arginine and *L. plantarum*, LI was reduced, in accordance with our previous findings.[20] The effects of arginine administration

Table 3. ILEAL, CECAL AND COLONIC MUCOSAL NUCLEOTIDES, RNA AND DNA

	Ileum			Cecum			Colon		
Groups	Nu.	RNA	DNA	Nu.	RNA	DNA	Nu.	RNA	DNA
Normal	2.9 ± 0.2	7.2 ± 0.2	8.1 ± 0.3*	3.0 ± 0.4	5.6 ± 0.6	8.7 ± 1.7†	2.6 ± 0.2	5.4 ± 0.2	7.3 ± 0.2
Liver injury LI	3.8 ± 0.2†	5.8 ± 0.6†‡	6.9 ± 0.7*	2.8 ± 0.2	4.4 ± 0.4	5.6 ± 0.7*†	2.5 ± 0.1	4.7 ± 0.3‡	6.3 ± 0.4*
LI + L-NAME	3.5 ± 0.3†	4.4 ± 0.3†§	6.1 ± 0.5*	2.5 ± 0.2	4.8 ± 0.3	6.5 ± 0.4*	2.9 ± 0.4	3.7 ± 0.2†	6.9 ± 0.7*
LI + Arginine	3.5 ± 0.1†	6.3 ± 0.4‡	7.6 ± 0.4*	2.9 ± 0.2	4.8 ± 0.2	6.1 ± 0.3*	2.8 ± 0.3	5.5 ± 0.2‡	6.5 ± 0.3*
LI + Arginine + L-NAME	2.3 ± 0.1*‡‖¶	5.7 ± 0.3†	6.7 ± 0.3*	2.2 ± 0.1	5.3 ± 0.1	6.4 ± 0.2*	2.3 ± 0.1	5.3 ± 0.1‡	6.7 ± 0.2*
LI + Arginine + Lb. Plantarum	3.0 ± 0.1‖	6.7 ± 0.3	8.1 ± 0.6*	2.4 ± 0.1	5.0 ± 0.1	7.1 ± 0.2*	2.2 ± 0.2	4.7 ± 0.1‡	6.8 ± 0.2*
LI + Arginine + Lb. Plantarum + L-NAME	2.7 ± 0.2*‡‖¶	5.1 ± 0.3†§	7.3 ± 0.7*	2.7 ± 0.1	5.5 ± 0.2	8.2 ± 0.6	2.4 ± 0.2	4.4 ± 0.3¶	7.2 ± 0.7*
LI + Lb. plantarum	3.6 ± 0.1†	5.5 ± 0.3†	10.4 ± 0.8	2.5 ± 0.2	5.1 ± 0.2	10.1 ± 0.5	2.6 ± 0.1	4.6 ± 0.4‡	9.5 ± 0.9†
LI + Lb. Plantarum + L-NAME	2.6 ± 0.2*‡	5.4 ± 0.2†	8.5 ± 0.6*	2.5 ± 0.1	5.8 ± 0.2‖	9.0 ± 0.6‖	2.6 ± 0.1	4.9 ± 0.2‡	8.6 ± 0.7

* $p < 0.05$ compared with LI + *Lb. plantarum* group using pairwise multiple comparison Newman-Keuls method.
† $p < 0.05$ compared with normal control.
‡ $p < 0.05$ compared with LI + L-NAME group.
§ $p < 0.05$ compared with LI + Arginine + *Lb. plantarum* group.
‖ $p < 0.05$ compared with liver injury group.
¶ $p < 0.05$ compared with LI + Arginine group.

could be almost completely abolished by the addition of L-NAME. These findings indicate that nitric oxide has a role in the protective effect of arginine on the liver, and that other mechanisms of action are less likely.

The administration of arginine and *L. plantarum*, alone or in combination, significantly reduced the level of liver enzymes compared with all the groups in which L-NAME was added. Some *Lactobacillus* strains can catabolize arginine to citrulline, using the arginine dihydrolase enzyme,[15,29] which is the same pathway used for nitric oxide formation.[30,31] We have previously shown that *L. plantarum* does not possess the enzyme arginine dihydrolase, according to the API 20 strep test;[20] however, another *L. plantarum* strain has been shown by others to be unable to degrade any amino acids other than tyrosine and arginine.[32] Rectal administration of *L. plantarum* seemed to reduce LI to a greater extent than administration of arginine, but the effects of either substance in isolation were abolished by L-NAME, indicating a role for nitric oxide.

The mechanism underlying the protective effect of *L. plantarum* on the liver is not obvious. There could be an augmentation of the systemic immune response and stimulation of the intestinal mucosal immune system. The effects could also be mediated by stimulatory effects on the intestinal mucosa, alteration of the intestinal microflora, and effects on the intestinal barrier function. When arginine and *L. plantarum* were administered together, the greatest protection was achieved, and this effect was not abolished by L-NAME. This may indicate that they have a synergistic action that is partially independent of nitric oxide. We did not investigate whether the effect of L-NAME is dose-dependent. The dose we used was selected on the basis of other studies concerned with liver function and nitric oxide. It is important to know whether this effect is completely abolished by the addition of higher concentrations of L-NAME.

A recent study showed that L-NAME is hepatotoxic.[33] The greater liver damage caused by L-NAME (the inhibitor of all NOS forms) compared with S-methylisothiourea (selective inhibitor of iNOS) could be the result simply of inhibition of the cNOS and a consequent decrease in nitric oxide production, and not the result of the assumed direct hepatotoxicity of L-NAME.

The administration of *L. plantarum*, with or without arginine, significantly reduced bacterial translocation. These results are in accordance with our previous findings.[20] The addition of L-NAME to arginine increased bacterial translocation to the liver and mesenteric lymph nodes, indicating a role for nitric oxide in the effect of arginine on bacterial translocation. Arginine and *L. plantarum* administration also significantly decreased bacterial translocation compared with the LI and the LI + L-NAME groups. However, inhibition of nitric oxide production in the arginine and *L. plantarum* groups increased bacterial translocation, even though arginine is known to reverse the effects of L-NAME in a dose-dependent manner. It seems that reversal of the L-NAME effect by arginine is not complete, leading to some degree of nitric oxide inhibition. It is also possible that arginine is degraded by lactobacilli or other bacteria, thereby making it possible for L-NAME to inhibit nitric oxide production in the gut lumen and tissues. Lactic acid bacteria[16] and *Lactobacillus* strains[29–32] can degrade arginine. Inhibition of nitric oxide production in conjunction with *L. plantarum* administration increased bacterial translocation to the liver and mesenteric lymph nodes, indicating an indirect role for nitric oxide by inhibition of its beneficial effects on microorganisms, intestine, and liver. Nitric oxide is a potent mediator of antimicrobial defense mechanisms,[34–36] thus leading to an increase in the clearance of translocated bacteria. Nitric oxide production maintains liver tissue integrity,[37] thereby improving its capacity for clearance of translocated bacteria. It also maintains the integrity of the intestine,[38] and endogenous and exogenous sources of nitric oxide serve as physiologic cytoprotectants for the mucosa.[39] Inhibition of nitric oxide causes a rapid increase in mucosal permeability[40,41] and facilitates mucosal injury.[42,43] *L. plantarum* significantly reduced bacterial translocation compared with the LI group and all the groups with added L-NAME. The effects of *L. plantarum* on bacterial translocation could be mediated via maintenance of gastrointestinal epithelial proliferation and function, inhibition of the growth of potentially pathogenic bacteria, and activation and augmentation of the systemic immune response and intestinal mucosal immune system. We have previously shown that administration of *L. plantarum* enhances the gut immune function,[44] stimulates the gastrointestinal epithelial proliferation, and improves the mucosal barrier function.[14]

Contrary to our results on bacterial translocation, another recent study showed inhibition of NOS ameliorated lipopolysaccharide-induced translocation of bacteria and largely prevented the development of intestinal hyperpermeability.[45] Other studies, however, in agreement with ours, show that the inhibition of nitric oxide production is capable of increasing intestinal permeability.[40,41] The difference could result from the different NOS inhibitors used. Unno et al.[45] used the more selective iNOS inhibitors aminoguanidine and S-methylisothiourea, both of which also have some other pharmacologic actions. Lipopolysaccharide, however, is not a specific inducer of iNOS: it also upregulates the biosynthesis of many other inflammatory mediators. Accordingly, the data in their study are insufficient to provide unambiguous evidence that lipopolysaccharide-induced intestinal mucosal hyperpermeability is mediated by increased production of nitric oxide. The two studies also differ in the methods used for evaluating bacterial translocation. Unno et al. considered growth of any colony-forming unit as positive and studied only aerobic growth. They compared controls to all cultured organs together. In our study, we used the bacterial number rather than the mere incidence of translocation. We think that the number of the translocated bacteria more appropriately reflects bacterial translocation

than a single colony-forming unit. We compared the numbers of bacteria separately translocated to each organ cultured in both aerobic and anaerobic conditions.

The addition of L-NAME increased the cecal and colonic bacterial counts. These results generally strengthen the evidence for the effect of nitric oxide as an antimicrobial agent, and are supported by the fact that nitric oxide is a potent mediator of antimicrobial defense mechanisms.[34–36] *L. plantarum* decreased the level of *Enterobacteriaceae* in the colon, and this has also been seen in other studies.[14,20] This effect of *L. plantarum* could be one of the mechanisms by which it reduces bacterial translocation.

Although D-galactosamine is a selective hepatotoxin, there was some effect on the gastrointestinal mucosa, with decreased content of RNA and DNA. Inhibition of nitric oxide in LI had no significant effect on the mucosal nucleotide, RNA, and DNA content. Inhibition of nitric oxide in the arginine, arginine + *L. plantarum*, and *L. plantarum* groups significantly reduced the ileal nucleotides and RNA. Nitric oxide is known to maintain intestinal tissue integrity during the inflammatory process,[38] and the inhibition of nitric oxide synthesis facilitates mucosal injury.[42,43] Administration of *L. plantarum* increased the ileal mucosal nucleotides, RNA, and DNA and cecal and colonic DNA contents compared with all the other groups. Lactobacilli are known to be involved in host metabolism and to offer nutritional and therapeutic benefits,[6] and they may play a role in maintaining gastrointestinal epithelial proliferation and function.[46] This effect was not influenced by the inhibition of nitric oxide production.

The inhibition of nitric oxide modulated the effects of rectal administration of arginine and *L. plantarum* in this acute LI model. L-NAME exacerbated the LI and increased bacterial translocation and cecal and colonic bacterial counts. Nitric oxide plays a role in the effects of arginine on the extent of LI and bacterial translocation.

Inhibition of nitric oxide production potentiates LI and increases bacterial translocation in D-galactosamine-induced LI. The LI can be reduced by arginine administration, and this effect is mediated by nitric oxide. Rectal administration of *L. plantarum* reduces the LI to some extent, and this effect is mediated mainly by stimulation of intestinal mucosal proliferation, improvement of barrier function, and influence on intestinal microecology. This is not mediated by nitric oxide. The effect of arginine and *L. plantarum* on the liver and on bacterial translocation could be mediated through mechanisms other than the production of nitric oxide The results of the experiment point to the importance of a gut factor in LI. Thus, therapeutic measures in acute liver failure should be multifactorial and should be aimed at both the liver and the gut.

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