Ascorbic Acid Improves the Intrahepatic Endothelial Dysfunction of Patients With Cirrhosis and Portal Hypertension

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Patients with cirrhosis show intrahepatic endothelial dysfunction, characterized by an impaired flow-dependent vasorelaxation. This alteration is responsible for the marked postprandial increase in portal pressure and is attributed to an insufficient release of nitric oxide (NO). Ascorbic acid reverts endothelial dysfunction in other vascular disorders, via the increase of NO bioavailability through the neutralization of superoxide anions, thus preventing the scavenging of NO by superoxide. This study examined whether acute ascorbic acid administration might improve endothelial dysfunction in cirrhosis. Thirty-seven portal hypertensive patients with cirrhosis had measurements of hepatic and systemic hemodynamics, ascorbic acid, and malondialdehyde (MDA). Patients were randomly allocated to receive ascorbic acid (3 g, intravenously, n = 15) or placebo (n = 12) followed by a liquid meal. A third group received ascorbic acid followed by a sham meal (n = 10). Measurements were repeated after 30 minutes (hepatic venous pressure gradient at 15 and 30 minutes). Patients with cirrhosis had significantly lower ascorbic acid levels and higher MDA than healthy controls. Ascorbic acid significantly reduced MDA levels and markedly attenuated the postprandial increase in the hepatic venous pressure gradient (4% ± 7% vs. 18% ± 10% in placebo at 30 minutes, $P < .001$). Ascorbic acid followed by sham meal did not modify hepatic or systemic hemodynamics. In conclusion, patients with cirrhosis exhibited intrahepatic endothelial dysfunction, associated with decreased levels of ascorbic acid and increased levels of MDA. Ascorbic acid improved intrahepatic endothelial dysfunction, blunting the postprandial increase in portal pressure. These results encourage the performance of further studies testing antioxidants as adjunctive therapy in the treatment of portal hypertension. (HEPATOLOGY **2006;43:485-491.**)

Portal hypertension is a serious consequence of cirrhosis and can result in life-threatening complications with increased mortality and morbidity.[1] Portal hypertension is determined by an increased resistance to portal-collateral blood flow and aggravated by an increased portal venous inflow, caused by splanchnic vasodilatation.[2]

The primary factor in the pathophysiology of portal hypertension is increased resistance.[3] In cirrhosis, the increase in resistance occurs at the level of the hepatic microcirculation and is promoted by the morphological changes occurring in chronic liver diseases. In addition, the active contraction of different cell types that are able to constrict or relax in a reversible and graded manner in response to several stimuli promote a further increase or decrease in the intrahepatic resistance.[4]

Insufficient nitric oxide (NO) production is considered a major pathogenic factor increasing intrahepatic vascular tone in cirrhosis.[5-7] The increased vascular tone is

Abbreviations: NO, nitric oxide; eNOS, endothelial nitric oxide synthase; ROS, reactive oxygen species; $O^{\cdot}$, superoxide; HVPG, hepatic venous pressure gradient; CO, cardiac output; HBF, hepatic blood flow; MAP, mean arterial pressure; MDA, malondialdehyde.

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associated with impaired vasorelaxation. Thus, the liver with cirrhosis, unlike a normal liver, cannot accommodate a volume load, such as that caused by meals, which results in an abrupt postprandial increase in portal pressure.[8] Recent studies have shown that such increase can be attenuated by increasing hepatic NO delivery.[8,9] Altogether these data suggest an insufficient NO bioavailability as the cause of the impaired vasorelaxation in response to blood flow, which define what is known as endothelial dysfunction.

A decline in NO bioavailability may be caused by either decreased expression[10] or posttranslational regulation of endothelial NO synthase (eNOS),[5,7,11] deficiency of eNOS substrate or cofactors for eNOS activity,[12] or by accelerated NO degradation because of its interaction with reactive oxygen species (ROS).[13] In this regard, increased production of ROS[14] and reduced antioxidant defenses have been described in cirrhosis, resulting in increased oxidative stress.[15,16]

Ascorbic acid (vitamin C) is a potent antioxidant that has been consistently shown to improve NO-dependent vasodilatation in vascular beds of patients with conditions characterized by marked endothelial dysfunction, such as hypertension, diabetes, hypercholesterolemia, and coronary heart disease.[17-22] The beneficial effect of acute ascorbic acid administration has been attributed to its capacity to neutralize ROS, mainly superoxide ($O^{\bullet}$). This prevents NO scavenging by ROS, increasing NO bioavailability.[23,24]

The aim of this study was to investigate whether ascorbic acid administration might improve hepatic endothelial dysfunction and attenuate the postprandial increase in portal pressure in patients with cirrhosis and portal hypertension.

Patients and Methods

Patients. The study was performed in 37 patients with cirrhosis, referred to the Hepatic Hemodynamic Laboratory at the Liver Unit for evaluation of portal hypertension from May 2003 to November 2005. All patients had liver cirrhosis diagnosed by clinic, biological, ultrasonographic, or histological criteria.

Patients were considered eligible for the study if they were found to have a hepatic venous pressure gradient (HVPG) $\geq$ 12 mmHg during the hemodynamic study. Exclusion criteria were hepatic failure, defined as prothrombin rate $<$ 40% and bilirubin $>$ 5 mg/dL; pregnancy; portal vein thrombosis; cardiac, renal, or respiratory failure; previous surgical or transjugular intrahepatic portosystemic shunting; diffuse or multinodular hepatocellular carcinoma; prescription of vasoactive drugs, antioxidants, or any previous hypersensitivity to ascorbic acid.

The study was performed according to the principles of the Declaration of Helsinki (revision of Edinburgh 2000), and the protocol was approved by the Ethics Research Committee of the Hospital Clinic in April 2003. Informed written consent to participate in the study was obtained in each patient.

Methods. After fasting overnight, patients were transferred to the Hepatic Hemodynamic Laboratory. Under local anesthesia, an 8 F venous catheter introducer (Axcess; Maxxim Medical, Athens, TX) was placed in the right jugular vein under ultrasonographic guidance (SonoSite Inc, Bothell, WA) using the Seldinger technique. Under fluoroscopic control, a Swan-Ganz catheter (Edwars Laboratory, Los Angeles, CA) was advanced into the pulmonary artery for measurement of cardiopulmonary pressures and cardiac output (CO) by thermal dilution. A 7 F balloon-tipped catheter (Medi-Tech; Boston Scientific Cork Ltd., Cork, Ireland) was then advanced into the main right hepatic vein to measure wedged and free hepatic venous pressures as previously described.[8,9] Preceded by a priming dose of 5 mg, a solution of indocyanine green (Pulsion Medical Systems, Munich, Germany) was infused intravenously at a constant rate of 0.2 mg/min. After an equilibration period of at least 40 minutes, 4 separate sets of simultaneous samples of peripheral and hepatic venous blood were obtained for the measurement of hepatic blood flow (HBF) as previously described.[25] To avoid interferences from differences in plasma turbidity, the Nielsen correction was used.[26] Mean arterial pressure (MAP) was measured every 5 minutes by a non-invasive automatic sphygmomanometer (Marquette Electronics, Milwaukee, WI). Heart rate was derived from continuous electrocardiogram monitoring.

All measurements were performed in triplicate in each study period, and permanent tracings were obtained on a multichannel recorder (Marquette Electronics). Portal pressure was estimated from the HVPG, the difference between wedged and free hepatic venous pressure. The hepatic vascular resistance (dyne $\cdot$ s $\cdot$ cm^{-5}) was estimated as HVPG (mmHg) $\times$ 80/HBF (L/min).[8,9] The systemic vascular resistance (dyne $\cdot$ s $\cdot$ cm^{-5}) was calculated as MAP (mmHg) $-$ right atrial pressure, mmHg $\times$ 80/CO (L/min).

After completing baseline hemodynamic measurements, patients were randomly allocated to receive in double-blind conditions either ascorbic acid (Roche Farma, Barcelona, Spain. 3 g, intravenously in 100 mL saline during 15 minutes, n = 15) or placebo (100 mL saline solution 0.9%, n = 12), followed by a mixed liquid meal (400 mL) containing 26 g proteins, 74 g carbohy-

Table 1. Baseline Clinical, Hemodynamic, and Laboratory Data of the Patients Studied

	Ascorbic Acid/Sham Meal (n = 10)	Placebo/Test Meal (n = 12)	Ascorbic Acid/Test Meal (n = 15)
Sex (M/F)	5/5	6/6	9/6
Age (y)	60.2 ± 9.1	60.1 ± 8.5	60.8 ± 9.4
Ascites (n)	6	4	5
Varices (small/large)	8 (7/1)	9 (5/4)	13 (8/5)
Previous bleeding	1	3	4
Child score	7.5 ± 1.7	7.2 ± 2	6.8 ± 1.7
Albumin (g/L)	33 ± 6	34 ± 8	34 ± 6
Bilirubin (mg/dL)	1.5 ± 0.6	2 ± 1.3	1.5 ± 0.9
Prothrombin activity (%)	66 ± 15	65 ± 14	68 ± 13
HVPG (mmHg)	19.1 ± 4.2	18.8 ± 3.8	18.1 ± 4.3
HBF (mL/min)	853 ± 365	808 ± 396	707 ± 241
Ascorbic acid (μmol/L)	37.7 ± 13	36.9 ± 21.3	38.4 ± 16.7
Malondialdehyde (nmol/L)	60.4 ± 46	74.8 ± 41.6	68.9 ± 35.5
Peripheral NOx (nmol/mL)	33.7 ± 11	35 ± 10	39.4 ± 20.2
Hepatic NOx (nmol/mL)	33.5 ± 11	30.4 ± 9.7	38.5 ± 16.8

NOTE: Results are expressed as mean ± SD. There were no significant differences in any parameter.
HVPG, hepatic venous pressure gradient; HBF, hepatic blood flow; NOx, nitric oxide products.
Reference values: albumin 37–53 g/L; bilirubin 0.2–1.2 mg/dL; prothrombin activity 80%–100%; peripheral NOx 37 ± 14 nmol/mL.

drates, and 21 g lipids for a total of 613 kcal (85 g of Scandishake Mix, International SHS, Spain; plus 25 g Resource Protein Instant, Novartis, Spain and 16 g sucrose), which was ingested within approximately 5 minutes. The test meal used in the current study was a homemade equivalent to that used in our previous studies (Ensure plus®),[8,9] modified to be free of ascorbic acid and other antioxidants. The 3-g dose of ascorbic acid has been shown to revert endothelial dysfunction in other vascular disorders[20,22] by scavenging superoxide. A third group received ascorbic acid followed by a sham meal (400 mL water, n = 10) to assess its effects independently of the postprandial response. The systemic and splanchnic response to the test meal was evaluated at 30 minutes, when maximal postprandial hyperemia and increase in HVPG has been demonstrated to occur.[27-29] HVPG was also measured at 15 minutes.

Biochemical Measurements. Blood samples from a peripheral vein and from the hepatic vein were taken at baseline and 30 minutes after the liquid meal. Plasma was separated within 15 minutes and frozen at −70°C for subsequent analysis. In peripheral samples, ascorbic acid levels and the degree of serum oxidative stress measured as the reaction products of malondialdehyde (MDA) with thiobarbituric acid reactive substances, were evaluated by high-performance liquid chromatography (Waters chromatograph, Waters Corp., Milford, MA). NO products (NOx; NO_2^-, and NO_3^- from peripheral and hepatic vein) were measured by chemiluminescence (Nitric Oxide Analyzer, NOA 280; Sievers Instruments, Boulder, CO). The determinations were performed before and after administration of ascorbic acid. A peripheral blood sample was obtained for ascorbic acid and MDA determination from a control group of healthy subjects (n = 33) with no evidence of liver disease and normal laboratory profiles matched for age, sex, and body mass index (age, 60.4 ± 8.5 years; range, 41-75 years; sex: 15 females/18 males, body mass index: 25.9 ± 4.7 kg/m^2).

Statistics. Statistical analyses were performed using SPSS 11.0 statistical package (SPSS Inc., Chicago, IL). All results are expressed as mean ± SD values. Comparisons within each group were performed with Student *t* test for paired data, and comparisons between groups by ANOVA followed by pre-planned contrast analysis. Wilcoxon test was used when appropriate. Correlation was performed by means of Pearson′s coefficient. Statistical significance was established at $P < .05$.

Results

The baseline clinical, hemodynamic, and laboratory characteristics of the 37 patients are shown in Table 1. There were not statistically significant differences between the 3 groups.

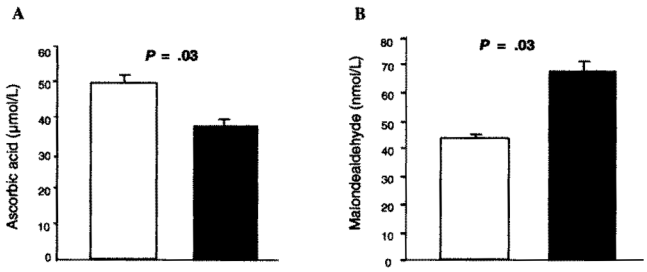

Fig. 1. Peripheral levels of ascorbic acid (A) and malondialdehyde (B) in healthy controls (white bars) and patients with cirrhosis (black bars) (error bars represent SEM).

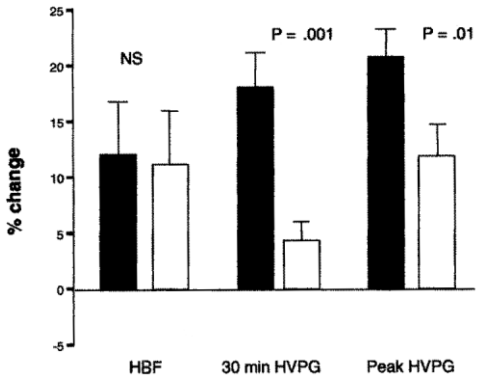

Fig. 2. Comparison of postprandial changes in hepatic blood flow (HBF) and hepatic venous pressure gradient (HVPG) at 30 minutes and peak HVPG between patients pretreated with placebo (black bars) or ascorbic acid (white bars) (data shown as mean % change from baseline ± SEM).

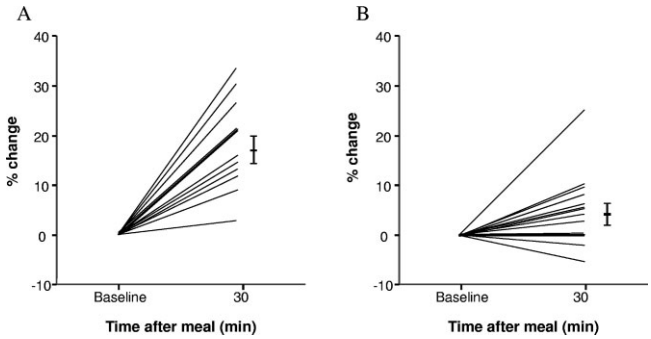

Fig. 3. Individual changes in hepatic venous pressure gradient 30 minutes after meal ingestion in the placebo (A) and in the ascorbic acid group (B). Bars indicate mean % change ± SEM.

Plasma ascorbic acid levels were significantly lower and plasma MDA levels higher in patients with cirrhosis as compared with healthy controls (37.6 ± 16.7 vs. 47.5 ± 18.4 μmol/L, $P = .03$, Fig. 1A; and 68 ± 40.2 vs. 44.5 ± 11.2 nmol/L, $P = .03$, Fig. 1B; respectively). Ascorbic acid levels significantly increased after ascorbic acid administration (from 38 ± 15 to 303 ± 78 μmol/L; $P < .001$) but did not significantly change after placebo (from 37 ± 21 to 44 ± 21 μmol/L; NS). No correlation was found between baseline ascorbic acid concentration and baseline splanchnic or systemic hemodynamic parameters. MDA levels significantly decreased after ascorbic acid administration (64.6 ± 40.2 to 50 ± 16.3 nmol/L, $P = .04$) but not after placebo (74.8 ± 41.6 to 67.5 ± 39.7 nmol/L, $P = .4$).

In patients receiving placebo, the test meal produced the expected significant increase in HBF (13% ± 15%, $P = .01$) and HVPG (12% ± 7% at 15 minutes, $P < .001$ and 18% ± 10% at 30 minutes, $P < .001$) (Fig. 2), mainly due to a marked increase in wedged hepatic venous pressure (WHVP) (Table 2). No significant changes in MAP, CO, systemic vascular resistance, and heart rate were observed after the test meal.

In patients receiving ascorbic acid, the test meal produced a percent increase in HBF similar to that observed in the placebo group (Fig. 2). However, the increase in HVPG was markedly attenuated as compared with that observed in patients receiving placebo (7% ± 11% vs. 12 ± 7%, $P = .2$ at 15 minutes; 4% ± 7% vs. 18% ± 10%, $P < .001$ at 30 minutes and 12% ± 9% vs. 21% ± 8%, $P = .01$ at peak value; Fig. 2). Figure 3 and Fig. 4 show the individual changes in HVPG and HBF after the meal, respectively. Estimated hepatic resistance was unchanged after meal in the ascorbic acid group (−4% ± 16%), but increased with placebo (6% ± 15%), this difference approaching statistical significance ($P = .10$).

No significant differences in hepatic NOx levels were observed after the test meal either in patients receiving placebo (from 30.4 ± 9.7 to 32.4 ± 10.8 nmol/mL; $P = .2$) or ascorbic acid (from 38.5 ± 16.8 to 38.8 ± 19.4 nmol/mL; $P = .8$). Peripheral levels were also not significantly modified (data not shown).

Patients receiving ascorbic acid followed by the sham meal did not experience any significant changes in hepatic or systemic hemodynamics and NOx (Table 3).

Table 2. Postprandial Changes in Splanchnic and Systemic Hemodynamics

	Placebo/Test Meal (n = 12)			Ascorbic Acid/Test Meal (n = 15)		
Variable	Baseline	30 min	*P* value	Baseline	30 min	*P* value
WHVP (mmHg)	27.6 ± 3.4	31.8 ± 3.3	<.001	27.3 ± 7	29 ± 8	<.001
FHVP (mmHg)	8.8 ± 3.5	9.7 ± 3.6	<.01	9.2 ± 4.9	10.1 ± 5.4	<.01
HVPG (mmHg)	18.7 ± 3.8	22 ± 3.8	<.001	18.1 ± 4.3	18.9 ± 4.9	<.001
HBF (mL/min)	808 ± 396	936 ± 537	.02	707 ± 241	771 ± 254	.04
MAP (mmHg)	91.6 ± 13	95.7 ± 14	*NS*	87.6 ± 10	89 ± 7.9	*NS*
CO (L/min)	7.4 ± 1.3	7.5 ± 1.3	*NS*	6.4 ± 1.4	6.7 ± 1.4	*NS*
SVR (dyne · s · cm^{-5})	970 ± 236	1003 ± 212	*NS*	1105 ± 414	1082 ± 320	*NS*
HR (bpm)	74 ± 9	79 ± 11	*NS*	81 ± 22	85 ± 21	*NS*

NOTE: Results are expressed as mean ± SD.

WHVP, wedged hepatic venous pressure; FHVP, free hepatic venous pressure; HVPG, hepatic venous pressure gradient; HBF, hepatic blood flow; MAP, mean arterial pressure; CO, cardiac output; SVR, systemic vascular resistance; HR, heart rate; *NS*, not significant.

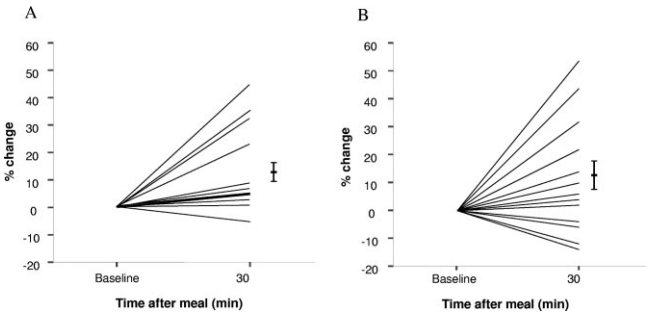

Fig. 4. Individual changes in hepatic blood flow 30 minutes after meal ingestion in the placebo (A) and in the ascorbic acid group (B). Bars indicate mean % change ± SEM.

Discussion

In cirrhosis, increased resistance to portal blood flow is determined by the morphological changes occurring in the liver and further aggravated by an increased hepatic vascular tone.[2,4] This latter component, which results from an insufficient hepatic bioavailability of NO[5,30] and an increased production of circulating and local vasoconstrictors (angiotensin, endothelin, cysteinyl-leukotrienes, thromboxane, and prostaglandins, among others),[31-35] is theoretically amenable to treatment with vasodilators.[36]

Attempts to correct the intrahepatic NO deficiency in experimental cirrhosis have involved NOS overexpression by transfecting the liver with adenovirus encoding eNOS, nNOS, or constitutively active AKT[10,37,38] or by selective NO donors.[39,40] In humans this has been attempted by the administration of low doses of isosorbide-5-mononitrate[8] or by modulating the post-translational regulation of eNOS by simvastatin.[9] However, the strategy of increasing NO bioavailability by reducing its degradation has not been addressed so far.

For over a decade NO has been known to be inactivated by ROS, particularly superoxide ($O^{\cdot}$).[41] Indeed, NO interacts with $O^{\cdot}$, and this reaction impacts directly on NO bioavailability. Under physiological conditions, endogenous antioxidant defenses minimize this interaction and maintain a balance between ROS and NO. However, this balance may be altered in a variety of disorders that show increased oxidative stress, such as hypertension, diabetes, hypercholesterolemia, and coronary heart disease,[42] leading to an impaired endothelium-dependent vascular relaxation. In these vascular disorders, the acute administration of the antioxidant ascorbic acid has proved effective at reverting endothelial dysfunction[17-22] because of its capacity for scavenging $O^{\cdot}$, which increases the bioavailability of endothelium-derived NO.[23,24]

In liver disease, firm evidence exists of enhanced oxidative stress. Much of it is derived from studies showing increased plasma and tissue levels of markers of lipid peroxidation[15,16,43,44] and from the observation of reduced hepatic and plasma antioxidant content.[45-47] Therefore, the intrahepatic NO deficiency might be, at least in part, attributable to the excess of $O^{\cdot}$ scavenging NO.

Our study confirms that patients with cirrhosis, similar to what occurs in patients with other chronic diseases with enhanced oxidative stress,[42] have reduced defensive mechanisms against oxidative stress, as indicated by the significant reduced levels of ascorbic acid and the increased levels of MDA, an index of lipid peroxidation. More importantly, the current study shows that the acute administration of high doses of the antioxidant ascorbic acid effectively attenuates the postprandial increase in portal pressure without causing any adverse effect. Because a similar increase in HBF was shown in patients receiving ascorbic acid or placebo, and given the trend toward a different response in the estimated hepatic resistance, we speculate that this effect might be attributable to ascorbic acid modulating hepatic vascular resistance. However, it should be stressed that, because accurate methods are not available for use in human patients to evaluate the resistance to portal blood flow generated by the liver,[2] the relation between HBF and HVPG provides only a rough estimate of the real hepatic resistance, and no definitive statements on this issue can be made.

The attenuation in the postprandial increase in portal pressure was associated with a reduction in MDA levels, strongly suggesting that ascorbic acid was exerting a potent antioxidant effect, therefore reducing $O^{\cdot}$ formation and NO scavenging. In addition, ascorbic acid may increase eNOS activity by different mechanisms, such as preventing the oxidation of tetrahydrobiopterin,[48,49] an essential eNOS cofactor. However, this is more likely to

Table 3. Systemic and Splanchnic Hemodynamic and Laboratory Changes After Ascorbic Acid Administration

Variable	Ascorbic Acid (n = 10)		
	Baseline	30 min	*P* value
WHVP (mmHg)	28.6 ± 5	29 ± 5.2	*NS*
FHVP (mmHg)	9.4 ± 3.6	10 ± 3.7	*NS*
HVPG (mmHg)	19.1 ± 4.2	18.9 ± 5.3	*NS*
HBF (mL/min)	853 ± 365	824 ± 293	*NS*
MAP (mmHg)	96.5 ± 15.5	98.7 ± 12.8	*NS*
CO (L/min)	7.8 ± 1.5	7.5 ± 1.8	*NS*
SVR (dyne · s · cm^{-5})	985 ± 353	1,069 ± 434	*NS*
HR (bpm)	80 ± 9	79 ± 9	*NS*
Hepatic NOx (nmol/mL)	33.5 ± 11	37.2 ± 8	*NS*
Peripheral NOx (nmol/mL)	33.7 ± 11	36.7 ± 10	*NS*

NOTE: Results are expressed as mean ± SD.

WHVP, wedged hepatic venous pressure; FHVP, free hepatic venous pressure; HVPG, hepatic venous pressure gradient; HBF, hepatic blood flow; MAP, mean arterial pressure; CO, cardiac output; SVR, systemic vascular resistance; HR, heart rate; NOx, nitric oxide products; *NS,* not significant.

occur after long-term ascorbic acid administration.[49] The lack of increase in hepatic venous NOx levels argues against increased NO production after acute ascorbic acid treatment, and supports the concept that the observed effects were the consequence of preventing NO from being scavenged by $O^{\cdot}$. Indeed, increased NO bioavailability is only followed by an increase in NOx when it is attributable to an increase in NO biosynthesis.[50]

The lack of effect of ascorbic acid in basal, non-stimulated HVPG is not surprising, taking into consideration that this is also not observed in other vascular beds, where ascorbic acid is known to improve endothelial dysfunction. This is usually tested by observing the effects of ascorbic acid on flow-mediated vasodilation[20,21] and after drug infusion of endothelium-dependent vasodilators such as acetylcholine.[17-19,22] Moreover, previous studies from our laboratory have shown that low doses of the NO-donor isosorbide-5-mononitrate had no effect on baseline HVPG but markedly attenuated postprandial increase in HVPG.[8] This is similar to that reported in experimental cirrhosis using the liver-specific NO donor NCX-1000.[40]

In conclusion, the current study demonstrates that patients with cirrhosis have increased MDA and decreased ascorbic acid levels, and that the acute administration of this antioxidant markedly attenuates the postprandial increase in portal pressure. Our findings suggest that increased oxidative stress may contribute to intrahepatic endothelial dysfunction in these patients, and that antioxidant therapy may counteract this abnormality. The results of the current study open the possibility of exploring antioxidants as adjunctive therapy in the medical treatment of portal hypertension.

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