Vitamin E supplementation reverses renal altered vascular reactivity in chronic bile duct-ligated rats

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Alcaraz A, Iyú D, Atucha NM, García-Estan J, Ortiz MC. Vitamin E supplementation reverses renal altered vascular reactivity in chronic bile duct-ligated rats. Am J Physiol Regul Integr Comp Physiol 292: R1486–R1493, 2007. First published December 7, 2007; doi:10.1152/ajpregu.00309.2006.—An altered vascular reactivity is an important manifestation of the hemodynamic and renal dysfunction during liver cirrhosis. Oxidative stress-derived substances and nitric oxide (NO) have been shown to be involved in those alterations. In fact, both can affect vascular contractile function, directly or by influencing intracellular signaling pathways. Nevertheless, it is unknown whether oxidative stress contributes to the impaired systemic and renal vascular reactivity observed in cirrhosis. To test this, we evaluated the effect of vitamin E supplementation (5,000 IU/kg diet) on the vasoconstrictor and vasodilator responses of isolated perfused kidneys and aortic rings of rats with cirrhosis induced by bile duct ligation (BDL), and on the expression of renal and aortic phosphoextracellular regulated kinase 1/2 (p-ERK1/2). BDL induced a blunted renal vascular response to phenylephrine and ACh, while BDL aortic rings responded less to phenylephrine but normally to ACh. Cirrhotic rats had higher levels of oxidative stress-derived substances [measured as thiobarbituric acid-reactive substances (TBARS)] and NO (measured as urinary nitrite excretion) than controls. Vitamin E supplementation normalized the renal hyporesponsiveness to phenylephrine and ACh in BDL, although failed to modify it in aortic rings. Furthermore, vitamin E decreased levels of TBARS, increased levels of NO, and normalized the increased kidney expression of p-ERK1/2 of the BDL rats. In conclusion, BDL rats showed a blunted vascular reactivity to phenylephrine and ACh, more pronounced in the kidney and reversed by vitamin E pretreatment, suggesting a role for oxidative stress in those abnormalities.

Cirrhosis; Kidney; Nitric oxide; Oxidative stress; Phosphoextracellular regulated kinase 1/2

Chronic bile duct ligation (BDL) induces intense changes in the circulatory and renal function, such as a fall in blood pressure and sodium retention, leading to the development of ascites and the hepatorenal syndrome with the advance of the disease (21, 24). At this point, the therapeutic options are complicated and very limited. Recently, several investigations have been proposed to examine whether oxidative stress is implicated in those alterations, so that more effective therapeutic tools could be developed. This is due to the fact that oxidative stress levels are increased in plasma and urine from patients and rats with liver disease (10, 31, 37–38) and can reach extremely high concentrations in patients with hepatorenal syndrome (34, 36), a condition characterized by profound renal sodium and water retention. Moreover, antioxidants may delay the development of a hyperdynamic circulatory state during experimental cirrhosis (17, 32) and improve renal dys-

function (1, 25, 41). However, it is unknown whether antioxidants can also prevent the altered systemic and renal vascular reactivity induced by liver cirrhosis.

Oxidative stress involves the generation of reactive oxygen species (ROS; O2 \textsuperscript{–}, H2O2, OH\textsuperscript{–}), reactive nitroxy species (peroxynitrite), and degradative products of lipid peroxidation (lipid peroxides, malondialdehyde, isoprostanes), which are used to measure oxidative stress levels (1, 17, 25, 31–32, 34–38, 41). These substances can affect vascular reactivity by several ways. They can disrupt lipid membranes, react with other molecules, and bind to receptors (11, 29). Thus, superoxide anion (O2 \textsuperscript{–}) can quickly react with nitric oxide (NO), further producing new oxidative-derived substances such as peroxynitrite (7), which can potentially modify the vascular function (8, 11, 48). But they can also regulate intracellular signaling molecules, such as the extracellular regulated kinase 1/2 (ERK1/2), which is implicated in contractile functions of vascular cells under physiological (22, 28) and pathological conditions (26, 30, 44, 47), although its role remained unclear under liver cirrhosis. Therefore, we hypothesized that during biliary cirrhosis, oxidative stress contributes to the systemic and renal excretory disturbances by altering vascular reactivity in the systemic and renal circulations. In addition, we evaluated whether the activation of the extracellular regulated kinase ERK1/2 is implicated in those alterations. To test this, we administered a high dose of the antioxidant vitamin E to rats 1 wk before inducing the biliary cirrhosis and then studied the vascular reactivity of aortic rings and isolated kidneys, NO levels, and the activation of ERK1/2.

Materials and Methods

Animals and Models

We used male Sprague-Dawley rats (225–250 g) born and raised in the animal facilities of the Universidad de Murcia (Murcia, Spain). All of the experimental protocols of this study were performed according to the Spanish Ministerio de Agricultura, Pesca y Alimentación, and the European Community guidelines for the use of experimental animals and approved by the Ethics Committee of the Universidad de Murcia.

Liver cirrhosis was induced by chronic BDL, a method frequently used in our laboratory (39, 41). Briefly, rats were subjected to either BDL or sham surgery. To that end, the rats were anesthetized with an intramuscular injection of a mixture of xylazine (50 mg/kg body wt im; Rompun, Bayer, Spain) and ketamine (100 mg/kg body wt im; Imalgène, Merial, France). Then, the bile duct was exposed under aseptic conditions by a midline incision under the sternum, doubly ligated with a 2–0 silk suture, and excised between the ligatures. For sham or control surgery, the bile duct was exposed and dissected but not ligated. After being killed, the rats were perfused with ice-cold saline at 100 mm Hg pressure. The liver, kidneys, and spleen were excised, weighed, and homogenized in 10 vol of ice-cold saline for NO determination and protein determination.

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not ligated or cut. All rats were maintained under comparable conditions with ad libitum diet and free access to drinking water. All of the experimental protocols were performed 21 days after surgery.

Study Design

Rats were randomized into three groups: 1) untreated control rats (control group; n = 19), 2) untreated BDL rats (BDL group; n = 24), and 3) BDL rats treated with vitamin E (BDL+VitE group; n = 22). A time control group of rats treated with vitamin E (Sham+VitE group; n = 4) was also included in the study. Both untreated groups were maintained on standard rat chow containing a normal essential amount of vitamin E (50 IU/kg diet). Group 3 received a diet supplemented with vitamin E (5,000 IU/kg diet, dry vitamin E acetate; Panlab S. L., Barcelona, Spain) starting 1 wk before surgery (day 0). All of the rats were acclimated to individual metabolic cages to collect 24-h urine samples, to determine urinary nitrites excretion. These were obtained during two consecutive days before surgery (days 2 and -1) and 2 days just before the experimental day (days 20 and 21 after surgery).

Experimental Procedure

Preparation of isolated perfused kidneys and functional procedures.

The rats were anesthetized and placed on a heated table to maintain body temperature at 37°C. A polyethylene cannula (PE-50) was placed in the right femoral artery for blood collection and for measuring blood pressure. Subsequently, we proceeded to isolate and perfuse the kidney, as described previously (19). Briefly, the left kidney was exposed by a midline laparotomy, and the renal artery was cannulated via suprarenal aorta to prevent or minimize interruption of blood flow. The kidney was perfused in situ with warm oxygenated Krebs’ buffer (in mM): 118 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 25 NaHCO3, 1.2 KH2PO4, 0.026 edetate calcium disodium, and 5.6 glucose, pH 7.4, at 37°C, blood-free and at a constant rate of 5 ml/min minus 1 kg weight -1 with a peristaltic pump (Master-flex 7518 – 00, Coler-Parmer Instrument, Niles, IL). The left renal vein was then cut and the ureter transected to allow exit of the perfusate. Finally, the kidney was excised from the surrounding tissues, decapsulated (to minimize any influence of interstitial pressure changes on renal vascular responses), and placed in a chamber containing Krebs solution at 37°C and the perfusate lost from the renal vein, both continuously drawn out of the chamber at the same rate of perfusion. Renal vascular responses were recorded through a transducer connected to a Macintosh LCII computer and analyzed with MacLab software (AD Instruments, Oxford, UK), as changes in renal perfusion pressure (RPP) downstream from the pump. Then, the right kidney and the thoracic aorta were removed and frozen for later studies. The weight of the right kidney was used as an index of the left kidney weight and the spleen weight as a reference of portal hypertension presence.

Renal responses to phenylephrine and ACh. After a 30-min stabilization period, we performed a dose-response curve to increasing doses of phenylephrine (PHE; 10-7, 5.10-6, 10-6, 5.10-5, 10-5, 5.10-4, and 10-4 M) administered as a 0.1-m1 bolus. The approximate time between doses was 2 min, the estimated time to reach the plateau with each dose, and similar to the required time to obtain a steady-state response by using a continuous infusion. In a subset of rats from groups 1, 2, and 3, the experiment was performed in the presence of N0-nitro-L-arginine methyl ester (L-NAME; 10-4 M) to inhibit NO synthesis. Thus, the drug was added to the Krebs solution 30 min before the initiation of the PHE dose-response curve and maintained it during the rest of the experiment.

Following the PHE curve and after another 30-min stabilization period, the renal vasculature was preconstricted with PHE (10-6 – 10-4 M) to reach a 75% of the maximal constriction observed in the previous curve. Once a stable elevated perfusion pressure was reached, the vasodilator responses to ACh (10-6 to 10-4 M) and to sodium nitroprusside (SNP; 10-6 to 10-4 M) were determined in all groups to assess NO and endothelium-dependent or -independent vasodilator responses, respectively. Changes in RPP in response to vasodilators are expressed as a percentage of the vasconstriction obtained with PHE.

Preparation of aortic rings and functional procedures.

A different set of animals was used for these experiments. After anesthesia, thoracic aortas were dissected free and cut into 3-mm rings in warm Krebs solution. Aortic rings were placed in 10-ml organ chambers to analyze vascular reactivity, as previously described (39). After equilibration, a cumulative concentration-response curve for PHE (10-6 to 10-3) was obtained. After repeated washing, rings were again constricted at 75% of the PHE maximal contraction to perform the cumulative concentration-response curve to ACh (10-6 to 10-4 M). L-NAME (10-4 M) was also used in a subgroup of rats from groups 1, 2, and 3. The presence of functional endothelium was confirmed after obtaining at least a 70% relaxation to ACh. In the L-NAME-treated rings, SNP (10-4 M) was used to test the functionality of aortic smooth muscle. Relaxation to ACh was expressed as a percentage of the maximal PHE effect. Washout solutions of these drugs were prepared in distilled water and maintained frozen. Working solutions were prepared daily in Krebs solution. Drug concentrations are expressed as final bath concentrations.

Analytical Measures

Thiobarbituric acid reactive substances (TBARS) were determined in plasma and kidney tissue, as a measure of lipid peroxidation, by a colorimetric method used previously (41). Urinary excretion of nitrates was determined by using the Griess reaction (40) in 24-h urine samples.

Renal and Aortic Expression of Phospho-ERK1/2

Western blot analysis of aortas and kidneys were performed following standard procedures, as described previously (20). Frozen aortas and kidneys were homogenized in a lysis buffer containing 150 mM NaCl, 5 mM EDTA, 20 mM Tris-HCl, supplemented with detergents (0.5% Igepal CA-630 and 1% Triton-X) and a cocktail of protease inhibitors (aprotinin 2 μg/ml, pepstatin A 1 μg/ml, leupeptin 10 μg/ml, 500 μM Na3VO4, and 1 mM PMSF). The homogenate tissue was spun down (10,000 g, 20,000 rpm) at 4°C, the pellet discarded, and the supernatant kept as tissue lysate. The protein concentration was measured in the lysates using a bichinoninic acid method (Sigma, St. Louis, MO). Protein samples (50–100 μg) were mixed (1:1) in 2× sample buffer (2% 2-beta-mercaptoethanol, 4% SDS, 20% glycerol, 0.001% bromophenol blue, and 500 mM Tris-HCl, pH 7.4) and boiled for 5 min. Then, proteins were separated by electrophoresis at constant voltage (100 V) on a polyacrylamide-SDS gel (11%) in a 2× Tris Base/192 mM Glycine/0.1% SDS buffer. Proteins were transferred to a 0.4-μm polyvinylidene difluoride membrane (Millipore) by wet electroblotting in a 25 mM-Tris Base/192 mM-Glycine non SDS-Glycine buffer. Nonspecific binding was blocked for 2 h in a Tris-buffered saline-Tween (TBS-T) blocking buffer (20 mM Tris Base, 500 mM NaCl, pH 7.5, 0.1% Tween 20) with 3% BSA. Western blot analysis was performed with specific monoclonal antibody anti-phospho-ERK1/2 (p-ERK1/2; BD Transduction). Blots were incubated in TBS-T-1% BSA with the primary antibodies (1:1,000) overnight at 4°C and washed. Then, the blots were incubated for 1 h in TBS-T-1% BSA with the secondary antibody (anti-mouse IgG, HRP-linked; 1:1,000) and washed. Prestained protein markers (Bio-Rad, Hercules, CA) were used for molecular weight determination. Lysates from A431 cells stimulated by EGF were used as positive control for p-ERK1/2. Both protein markers and positive control were run in parallel to the samples. Detection of specific proteins (4244 kDa) was carried out by enhanced chemiluminescence (ECL; Western blotting analysis system, Amersham, Little Chalfont, Buckinghamshire, UK). The bands corresponding to the different proteins in the ECL films were scanned, and the relative expression of protein was quantified by densitometric analysis. Densitometric results are reported as integrated values (area × times density of the band, corrected by protein loading) and expressed as a percentage.
compared with controls (100%). To determine protein loading, we stained the blots with a 0.1% of Ponceau Red solution. All drugs were purchased from Sigma Chemical (Madrid, Spain) except where indicated.

Histologic Analysis

Liver tissue samples were fixed in 10% buffered formaldehyde and then processed and embedded in paraffin and sectioned (~5 μm), as previously reported (15, 41). These sections were then stained with hematoxylin and eosin and viewed by light microscopy. A pathologist then performed morphologic evaluation in blinded randomized sections of the liver tissue.

Statistical Methods

Data are presented as means ± SE. Differences between groups were compared by one-way, repeated-measures ANOVA. For the dose-response curves, we applied a two-way repeated-measures ANOVA, and when significantly different, means were further compared by the Newman-Keuls test. The ED50 (concentration of agonist producing 50% of the maximal response) is derived from logarithm transformation and regression analysis of each individual concentration-response curves. Statistical significance was considered at P < 0.05.

RESULTS

General Parameters

BDL rats had significant lower body weight (244 ± 11 vs. 322 ± 6 g) and hematocrit (39.2 ± 1.2 vs. 45.6 ± 0.8%) than controls and higher spleen (1.81 ± 0.11 vs. 0.86 ± 0.03 g) and kidney-to-body wt ratio (0.42 ± 0.01 vs. 0.32 ± 0.01), although similar kidney weights (1.04 ± 0.03 vs. 1.01 ± 0.03 g), respectively. Vitamin E treatment significantly increased body weight, hematocrit, and kidney weight (288 ± 8 g, 43 ± 1%, and 1.13 ± 0.03 g, respectively) in the BDL animals, without altering spleen weight and kidney ratio (1.81 ± 0.11 g and 0.40 ± 0.02 g, respectively). The Sham + VitE group exhibited slightly different values only in the body weight (344 ± 4 g) but similar hematocrit (45.8 ± 0.6%), spleen weight (0.80 ± 0.07 g), kidney-to-body wt ratio (0.35 ± 0.02), and kidney weight (1.19 ± 0.08 g) than the untreated control group.

Untreated BDL rats had lower blood pressure (BP) compared with controls (84 ± 3 vs. 122 ± 6 mmHg), and treatment with vitamin E significantly increased it (BDL + VitE, 100 ± 6 mmHg; Fig. 1, top). Basal RPP was also lower in the BDL rats than in controls (37 ± 3 vs. 66 ± 11 mmHg) and vitamin E normalized it (65 ± 8, Fig. 1, bottom). A similar pattern in basal RPP was observed in the 1-NAME subgroup (BDL, 29 ± 5; Control, 63 ± 10; and BDL + VitE, 48 ± 5 mmHg), in which nonstatistical differences were observed between groups after 1-NAME administration (43 ± 9, 72 ± 13, and 73 ± 12 mmHg, respectively). Chronic vitamin E administration did not modify either BP (115 ± 4 mmHg) or RPP (54 ± 4 mmHg) to the control rats.

Finally, histological analysis of all the BDL rat livers revealed an altered pattern of liver tissue structure compared with the control groups with a marked bile-duct proliferation and patchy necrosis.

Renal Vascular Responses in the Isolated and Perfused Kidneys

The administration of PHE induced dose-dependent increases in RPP (Fig. 2, top) that were significantly lower in the BDL rats, and normalized by vitamin E supplementation (left). The ED50 value (Table 1) was higher in the BDL group and also normalized by vitamin E. 1-NAME administration (right) eliminated the differences between groups in response to PHE, and the BDL + VitE group reached higher values than the untreated BDL, as well as greater ED50 and maximal response values (Table 1).

The response to ACh (Fig. 2, bottom, and Table 1) was reduced in the BDL kidneys, and treatment with vitamin E restored it (left bottom and Table 1). No differences were found between groups after 1-NAME administration (right bottom). The ED50 values for ACh responses were similar in all groups (Table 1). The Sham + VitE group showed a similar pattern of renal vascular responses to PHE and ACh than the control group, even though the PHE-ED50 value was a little different. SNP induced similar responses in all groups before and after 1-NAME treatment.

Vascular Responses in Aortic Rings

The response to PHE in aortic rings (Fig. 3, top and Table 2) was significantly lower in BDL (treated or not with vitamin E) than in controls, and the addition of 1-NAME to both BDL groups abolished the hyporesponsiveness to PHE. The ED50 value (Table 2) was similar in control and BDL rings and lower
in the BDL+VitE group. L-NAME significantly decreased ED₅₀ in control and BDL rings. In contrast, ACh induced similar relaxations without differences between groups (except for BDL vs. BDL+VitE) before or after L-NAME administration (Fig. 3, bottom). ED₅₀ and maximal values of ACh responses remained unchanged in all groups (Table 2). Both ED₅₀ values and the aortic vascular responses to PHE and ACh in the Sham+VitE group were comparable to the control group (Table 2). Finally, treatments or manipulation did not alter smooth muscle function, as SNP relaxed evenly and 100% of all groups of rings.

**Systemic and Renal Levels of ROS and NO (TBARS and 24-h Urinary Nitrites)**

Plasma levels of TBARS were increased in the BDL group and remained elevated after vitamin E supplementation (Fig. 4, top). Conversely, in the kidney tissue (Fig. 4, middle), vitamin E treatment decreased the elevated renal levels of TBARS in the BDL group. BDL rats had elevated 24-h urinary nitrite excretion, and administration of vitamin E further increased this value in BDL rats (Fig. 4, bottom).

**Aorta and Kidney Protein Expression of Phospho-ERK1/2**

The aortic expression of pERK1/2 (Fig. 5, top) had a tendency to be greater in BDL groups, although we did not find statistical differences between them. On the contrary, the renal expression of this protein (Fig. 5, bottom) was increased in the BDL group, and vitamin E treatment returned the expression to control values.

**DISCUSSION**

The main findings in the present study are that vitamin E administration to BDL rats corrects the lower RPP and renal vascular responses to PHE and ACh. These effects were associated with a decrease in kidney TBARS and increased excretion of urinary nitrates, suggesting a better availability of NO in the renal vascular bed. We also found that the increased renal expression of pERK1/2 return to control values in the group of BDL treated with vitamin E. These results are consistent with our previous study, in which we reported that vitamin E prevents renal dysfunction and improved hypotension induced by experimental BDL using in vivo anesthetized rats (41) and, furthermore, brings to light additional mechanisms by which vitamin E may exert this beneficial effect.
Fig. 3. Vascular reactivity in aortic rings to phenylephrine (top) and Ach (bottom) in the control (○), BDL (▲), and BDL + VitE (▼) group, in the absence (left) or presence (right) of L-NAME (10⁻⁴ M). †P < 0.05 vs. control group; †P < 0.05 vs. BDL group.

Table 2. ED₅₀ values and maximum contraction or relaxation to phenylephrine and Ach in aortic rings of the different experimental groups

<table>
<thead>
<tr>
<th>Aortic Rings</th>
<th>Phenylephrine</th>
<th>Ach</th>
</tr>
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<tbody>
<tr>
<td>Control (6)</td>
<td>5.62 ± 0.49</td>
<td>2.92 ± 0.13</td>
</tr>
<tr>
<td>Sham + VitE (4)</td>
<td>4.18 ± 0.57</td>
<td>2.19 ± 0.06</td>
</tr>
<tr>
<td>BDL (7)</td>
<td>4.40 ± 0.33</td>
<td>1.76 ± 0.10a</td>
</tr>
<tr>
<td>BDL + VitE (5)</td>
<td>3.52 ± 0.42*</td>
<td>1.75 ± 0.11a</td>
</tr>
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</table>

L-NAME

| Control (4)  | 1.96 ± 0.13a | 2.45 ± 0.14 |
| BDL (5)      | 2.23 ± 0.1b  | 2.13 ± 0.23 |
| BDL + VitE (5) | 3.29 ± 0.79  | 2.61 ± 0.19a |

Values are presented as means ± SE. Numbers in parentheses are number of animals per group. *P < 0.05 vs. control group; †P < 0.05 vs. BDL group. a,b,cP < 0.05 with respect to the control, BDL, and BDL + VitE basal groups, respectively.

Prolonged obstruction of the bile duct in the rat usually causes a cholestatic liver disease with hepatocellular damage and complicated by portal hypertension, ascites, and the presence of a hyperdynamic circulation that leads to progressive hypotension, renal dysfunction, and death. The evolution is variable and the rats died usually within 5–8 wk, depending on the strain used and even on the supplier (13, 15–16, 31–32, 41). After 21 days of BDL (4th wk), the animals used in the present study showed typical signs of cirrhosis, such as decreased growth, jaundice, and coluria, and a lower hematocrit. After death, the abdominal inspection revealed a small amount of ascites only in one or two rats of the BDL groups, but there was mesenteric edema, as well as an enlarged liver and spleen (indirect evidence of portal hypertension) in all of them. Morphologically, the livers had marked ductal proliferation, fibrosis, and patchy necrosis, and as we previously found (41), vitamin E treatment had small or no effect on the liver morphology, portal hypertension, or spleen weight. That is not entirely surprising since the vitamin E therapy is not addressing the primary insult, which is the surgical cholestasis. In addition, analyzing previous studies using different antioxidants that found a recovery in the systemic hemodynamic changes, we have observed that the improvement in liver function is minimal (17, 32, 41). In fact, there was no improvement in serum bilirubin or albumin, and very minor decreases in plasma aspartate aminotransferase levels or portal pressures. Thus, we think that the data overall suggest that the antioxidant therapies employed in the present and past studies are not effective (or at best only marginally effective) in preventing the progression of the liver disease.

Chronic obstructive jaundice is also associated with severe hypotension and a predisposition to acute renal failure that may be related to changes in renovascular responsiveness (14, 27). In this regard, reduced vascular responses to vasoconstrictors in different vascular beds have been reported in experimental models of portal hypertension with or without cirrhosis (3, 13, 19, 39). In this study, we also found a lower renal vascular reactivity to PHE in isolated kidneys from rats subjected to BDL compared with their controls. Moreover, basal RPP of the kidneys from BDL rats was also lower than in the control animals, suggesting a basal dilation of the renal vasculature of the BDL group. This decrease of basal RPP in the BDL kidneys cannot be due to the systemic hypotension exhibited by BDL rats because 1) the kidney was isolated from the animal and 2) the perfusion rate was the same as in the control kidneys. Likely, this lower than normal renal vascular resistance is due to intrarenal factors.

NO, which is synthesized in excess during cirrhosis, has been one of the main implicated factors in this alteration. Thus, NO synthesis inhibition increased the reduced vascular re-
responses in isolated kidneys (19) of rats with portal hypertension, as well as we found in the BDL model, indicating that elevated levels of NO are contributing to the renal vasodilation. In this regard, an interesting finding of our study is that the response to ACh in the BDL kidneys was severely attenuated, and inhibiting NO synthesis with L-NAME further blunted this response. These results suggested that a factor different than NO is affecting the kidney; alternatively, it is possible that the excess of NO or some NO-derived substance is being harmful to the endothelium relaxing function. In this way, Inan et al. (27) obtained similar results, and they suggested that the overproduction of NO may cause endothelial damage and/or desensitization of guanylate cyclase, which, in turn, may result in loss of endothelium-dependent and -independent vasorelaxation. This is important because an altered renal endothelial vasodilation may contribute to explain previous in vivo studies in cirrhotic animals showing a defective renal vascular and excretory response to maneuvers aimed at vasodilating the kidney (2, 4–5).

To further investigate this, we analyzed oxidative stress between other factors contributing to the altered vascular responses in cirrhosis. Oxidative stress is increased in patients and animals with cirrhosis, and its levels correlate with the...
degree of the disease (10, 31, 36–38). In addition, several studies have shown that antioxidants may prevent, correct, or improve the circulatory and renal anomalies in human (25) and experimental models of cirrhosis (1, 16–17, 32, 41), although the mechanisms by which this occurs are not completely clarified. In this respect, we found that supplementation with vitamin E to BDL rats corrected the altered renal vascular reactivity to both PHE and ACh. To our knowledge, this is the first experimental evidence showing that an antioxidant improves renal vascular reactivity to both vasoconstrictors and vasodilators in rats with liver cirrhosis. This effect on renal vascular reactivity might contribute to explain part of the beneficial actions of antioxidants described in this and previous studies. The effect of vitamin E in the cirrhotic kidney appears to be dual. First, acting as a scavenger of ROS, as we found a significant decrease in kidney TBARS in the BDL-vitamin E-treated animals; and second, increasing the availability of NO, as suggested by the increased levels of urinary excretion of nitrites in this group. Superoxide anion (O$_2^-$) can rapidly react with NO, thus decreasing the availability of NO and resulting in peroxynitrite formation (OONO$^-$), which can subsequently initiate again lipid peroxidation (7), thus completing a vicious circle where NO is continuously consumed. Then, antioxidant administration will break that circle by diminishing the interaction between ROS (O$_2^-$) and NO, thus increasing NO bioavailability.

However, vitamin E treatment alone did not affect the aortic BDL hyporesponse, and only after NOS inhibition, the aortic response to PHE was normalized in the cirrhotic rats and further increased if they were pretreated with vitamin E. These responses in the aortic rings may be related to the different contribution of NO in the vascular responsiveness, depending on the vascular bed studied (42, 46), as well as the different patterns of oxidative stress that may be found in different tissues (31, 38). Actually, the levels of TBARS in kidney tissue are higher than in plasma, suggesting that kidneys are specially affected by oxidative stress, and thus the effect of vitamin E supplementation is more pronounced in this tissue than systemically. In fact, during cirrhosis, several substances that are able to induce oxidative stress are particularly elevated or affect the kidney, such as the renin-angiotensin system, endothelin, and catecholamines (21, 31). It is also possible that TBARS are not sensitive enough to detect the improvement, although we found a similar behavior when measuring F$_2$-isoprostanes (41), one of the more sensitive markers of oxidative stress (35). Thus, we think that it is more likely that there is compartmentalization of the antioxidant efficacy, and renal oxidative stress may have been reduced without a reduction in the systemic levels. Furthermore, our results indicate that the BDL-induced changes in the vascular reactivity of the thoracic aorta are more dependent on NO, because only NOS inhibition normalized the response to PHE in the cirrhotic rats, and it was under this condition that we saw some effect of vitamin E.

Finally, we found that expression of pERK1/2 is increased in the kidney and aorta of BDL rats, and administration of vitamin E normalized it in the kidney but not in the aorta. These changes parallel the results obtained in the vascular reactivity study and the oxidant stress markers, suggesting that they might be related. ERK1/2 is an extracellular regulated kinase sensitive to oxidative stress and can influence the contractile function (9, 26, 30, 44, 47) and promote growth and/or survival in vascular cells (22, 28). An activation of ERK1/2 has been described in different models of hypertension and the degree related to the levels of blood pressure (47) and the mechanical stretch (26). Because our BDL rats have a reduced blood pressure and a systemic vasodilation, there is little probability that a blood pressure effect counts for the ERK1/2 activation and more probably is related to something common to these two diseases, a high oxidative stress. Besides, during cirrhosis, the activation of neurohormonal systems (angiotensin, endothelin, and catecholamines) induced by the hemodynamic changes can also contribute to a redox-sensitive phosphorylation of ERK1/2 (18). Thus, vitamin E would decrease ERK1/2 activation and restore vascular reactivity according to the improvement of oxidative stress markers, which occurs in the kidney but not in the aorta.

In addition, NO has been reported to activate the ERK1/2 pathway in very smooth muscle cells (VSMC), and this activation seems to be involved in the inhibition of VSMC proliferation induced by NO (6, 23). But also a NO overproduction might be driven by oxidant stress via a phosphoinositide 3-kinase/Akt and MAPK/extracellular signal-regulated kinase/ERK1/2 pathways (12, 43), which have been implicated in the endotoxin-induced vascular hyporeactivity (30) where NO and free radicals are greatly elevated. Then, during cirrhosis, the resulting disequilibrium between the unrestrained oxidative stress and NO, and their interactions, favor the activation of ERK1/2. Consequently, treating the cirrhotic animals with vitamin E restores the normal equilibrium, more clearly inside the kidney, contributing toward normalizing NO production and availability and thus the renal responses to PHE and ACh.

In conclusion, we have shown that vitamin E supplementation restores the abnormal renal vascular responses to PHE and ACh in BDL-induced cirrhotic rats. These effects were due to a decrease in oxidative stress-derived substances levels in the kidney and to a better availability of renal vascular NO. The changes in pERK1/2 expression suggest that this intracellular signaling pathway may be implicated in the contractile dysfunction of VSMC during chronic BDL and can also contribute to explaining part of the beneficial effects of vitamin E on renal vasculature.

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