Quercetin Administration Ameliorates Pulmonary Complications of Cirrhosis in Rats

Juliana Tieppo, Maríα J. Cuevas, Rafael Vercelino, Maria J. Tuñon, Norma P. Marroni, and Javier González-Gallego

Abstract

In the hepatopulmonary syndrome (HPS), a common complication of liver cirrhosis, pulmonary endothelial endothelin B (ETB) receptor overexpression, enhanced endothelial nitric oxide (NO) synthase (eNOS)-derived NO production, and increases in pulmonary inducible NO synthase (iNOS) and heme oxygenase (HO-1) are important factors in the development of vasodilatation. These changes may be influenced by redox-sensitive signaling pathways, including nuclear factor-κB (NF-κB). In this study, our aim was to evaluate the effects of the flavonoid antioxidant quercetin on the development of HPS in rats with common bile duct ligation (CBDL). Rats were divided into the following 4 groups: rats subjected to CBDL, Sham (rats subjected to simulated CBDL), quercetin-treated sham, and quercetin-treated CBDL. Quercetin (50 mg/kg) was administered for 2 wk starting on d 14 after surgery. Increased NO production, overexpression of iNOS, eNOS, HO-1, and ETB-receptor and activation of NF-κB were observed in lung of CBDL rats. Quercetin inhibited oxidative stress, NF-κB activation, and the expression of different pulmonary mediators involved in HPS. Quercetin also ameliorated liver injury and reduced the expression of hepatic endothelin-1 and HO-1 in untreated cirrhotic rats. Our findings suggest that quercetin administered after the onset of hepatic injury significantly ameliorates pulmonary complications in CBDL rats and that limitation of cirrhotic evolution contributes to this effect. J. Nutr. 139: 1339–1346, 2009.

Introduction

Patients with liver cirrhosis bear a considerable risk of a variety of complications, such as variceal bleeding, ascites, spontaneous bacterial peritonitis, encephalopathy, and hepatopulmonary syndrome (HPS) (1). HPS results when intrapulmonary vascular dilatation causes hypoxemia in the setting of liver disease or portal hypertension (2). This syndrome is found in 10–20% of patients with cirrhosis and its presence increases mortality (3).

Despite a dramatic improvement in our understanding of HPS, its pathogenesis remains incompletely understood and no medical therapies are available.

Chronic common bile duct ligation (CBDL) leading to biliary cirrhosis reproduces in rats the pulmonary physiological abnormalities of human HPS and serves as an experimental model of the disease (4). A series of alterations in the pulmonary microvasculature have been identified after CBDL and contribute to intrapulmonary vasodilatation. These changes include endothelin-1 (ET-1)-mediated endothelial nitric oxide (NO) synthase (eNOS) activation and NO production via increased endothelial endothelin B (ETB) receptors (5). Moreover, the accumulation and activation of intravascular macrophages lead to inducible NO synthase (iNOS)-derived NO and heme oxygenase (HO)-1-derived carbon monoxide (CO) production (6,7). These changes may be influenced by activation of several transcription factors, such as nuclear factor-κB (NF-κB). Thus, ET-1 increases the formation of NF-κB complexes and the stimulation of NF-κB DNA binding by ET-1 involves the ETB-receptor (8). Moreover, NF-κB activation stimulates gene expression of adhesion molecules, inflammatory cytokines, and enzymes such as iNOS, eNOS, and HO-1 (9,10). There is also evidence that oxidative damage may play a large role in the progression of this syndrome (11,12) and some antioxidants efficiently decrease hepatic fibrosis in CBDL animal models (13,14).
Quercetin (3,5,7,3′-4-pentahydroxy flavone) is the major flavonoid found in the human diet (15). A number of beneficial effects of quercetin on human health have been shown (16) and some studies have indicated an important role for quercetin in fighting the deleterious effects of reactive oxygen species and in the inhibition of redox-sensitive signaling pathways, including NF-κB, in several diseases (17,18). In previous research, we also demonstrated that quercetin increased the genomic stability in rats with HPS, probably due to its antioxidant properties (19). However, additional studies should be performed to better understand the mechanism of protection by quercetin in HPS. Therefore, the present study was undertaken to evaluate the effects of quercetin on the development of HPS in rats with CBDL and to test whether quercetin treatment would decrease oxidative stress, NF-κB activation, and gene expression of the different mediators involved in HPS.

Materials and Methods

**Materials.** Quercetin was purchased from Sigma Chemical. TaqMan primers and probes for iNOS (GenBank accession nos. D12520.1 and Rn00561646_m1), eNOS (GenBank accession nos. AB176831.1 and Rn02136234_s1), HO-1 (GenBank accession nos. BC091164.1 and Rn01536933_m1), ETB-receptor (GenBank accession nos. X75764.1 and Rn00569139_ml), collagen type I (GenBank accession nos. BC108298.1 and Rn01562721_ml), procollagen type IV (GenBank accession nos. BC089096.1 and Rn01482927_ml), transforming growth factor-β1 (TGFβ1) (GenBank accession nos. X52498.1 and Rn00572010_ml), α-smooth muscle actin (α-SMA) (GenBank accession nos. A06801.1 and Rn01759928_g1), ET-1 (GenBank accession nos. M6471.1 and Rn00561129_ml), and the housekeeping gene hypoxanthine phosphoribosyl-transferase (HPRT) (GenBank accession nos. M63983.1 and Rn01527840_m1) were derived from TaqMan Gene Expression assays (Applied Biosystems). NF-κB and specificity protein 1 (SP1) oligonucleotides were from Promega. NF-κB oligonucleotide was labeled with (γ-32P)ATP from GE Healthcare Bio-sciences. Antibody against ED1 was from Santa Cruz Biotechnology, antibody against α-SMA was from Abcam, and anti-β-actin antibody was from Sigma. EnVision reagent was purchased by Dako Cytomation and 3,3′-diaminobenzidine was from Vector Laboratories.

**Rats and treatments.** The study protocol was approved by the institutional Animal Care Committee of the Hospital de Clínicas de Porto Alegre and conforms to the Guide for the Care and Use of Laboratory Animals of the NIH. Male Wistar rats (Panlab) were caged at 24°C with a 12-h-light/dark cycle and free access to food (Parnab A04). The composition of the diet was: 15.4% protein, 2.9% fat, 60.3% carbohydrate, 3.9% fiber, 5.3% minerals, and 12% water. Four groups of rats were subjected to CBDL as previously described (20) and studied 4 wk after CBDL or a sham operation: an untreated sham-operated group (CBDL), and a quercetin-treated CBDL group (Sham-Q), an untreated sham-operated group (Sham), and a quercetin-treated sham-operated group (Sham-Q). Quercetin was suspended, immediately before administration, in 0.2% Tween aqueous solution. Groups of Sham and cirrhotic rats received daily a 50-mg/kg intraperitoneal injection of quercetin starting 14 d after surgery until d 28 (13), when the rats were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg) and killed by exsanguination. Tissues were collected and stored at −80°C.

**Liver tests, portal venous blood pressure, and gas measurements.** On d 28, blood was collected into a heparinized glass capillary from the retro-ocular sinus for the analysis of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, and direct bilirubin, which were measured using commercial kits (Boehringer Mannheim). The abdominal region was shaved for mid-ventral laparotomy and blood was collected from the abdominal aorta to measure arterial gases (20) using an automatic blood gas analyzer (ABL 700, Radiometer). The alveolar-arterial oxygen gradient was calculated as alveolar-arterial oxygen difference (AaO2) = 150 – (PaCO2/0.8) – PaO2, where PaCO2 is the partial pressure of carbon dioxide in the arterial blood and PaO2 is the partial pressure of oxygen in the arterial blood. Portal venous pressure was measured on a Wilson biniscr iptal polygraph (Wilson Medical Electronics).

**Histological and immunostaining analysis.** The anterior portion of the lower right lobe of the lung and liver tissue were collected and kept for 12 h in 10% formaldehyde solution. After paraffin embedding, lung tissue slides were stained using hematoxylin-eosin and liver tissue slides using hematoxylin-eosin and Sirius red. In the lung tissue, the alveolar capillaries were selected for computed morphometric evaluation by a microscope equipped with a digital camera and connected to an image capture board of a computer running the Image-Plus software.

**Immunohistochemistry.** Immunohistochemistry using polyclonal antibody α-SMA was performed as a marker of activated hepatic stellate cells. Each section was deparaffinized by xylene and rehydrated with graded alcohols. The sections were retrieved in a microwave oven (300W) in citrate buffer (pH 6.0) for 10 min at 100°C. Endogenous peroxidase activity was blocked by incubation of slides in absolute methanol containing 3% hydrogen peroxide for 10 min at room temperature. The sections were sequentially preincubated with 10% normal rabbit serum for 10 min at room temperature to block the possible background staining by the secondary antibody. They were then incubated with antibody α-SMA at its working dilution of 1:200. After 60 min at room temperature, they were treated with EnVision reagent for 30 min. They were washed 3 times with PBS after each incubation, and 3,3′-diaminobenzidine was employed as a chromogen. The nuclei were lightly counterstained with hematoxylin solution. The primary antibodies were replaced with PBS containing 0.1% bovine serum albumin as a negative control. Pathological findings were assessed by one of the authors who was unaware of the group allocations using a light microscope equipped with a digital camera and connected to an image capture board of a computer using the Image-Plus software.

**Biochemical analysis.** Lipoperoxidation was measured using the TBARS assay (21). Cytosolic superoxide dismutase (SOD) was assayed by rate of epinephrine auto-oxidation as previously reported (22). The Griess reaction was used for the quantification of nitrate plus nitrite (NOx) (23).

**Real-time quantitative RT-PCR.** Total RNA was extracted and reverse transcribed using a High-Capacity cDNA Archive kit (Applied Biosystems) (24). cDNA was amplified using TaqMan Universal PCR Master mix (Applied Biosystems) on a Step One Plus (Applied Biosystems). Each assay included a no-template control and an RT negative control. Relative changes in expression levels were determined using the 2−ΔΔCT method (25). The cycle number at which the transcripts were detectable was normalized to the cycle number of HPRT gene detection, referred to as ΔCT.

**Nuclear extracts and electrophoretic mobility shift assay.** Binding activity of NF-κB was determined in nuclear extracts of lung using electrophoretic mobility shift assay (EMSA) (26). Competition experiments were also conducted using a negative control (cold probe) and lung sample plus nonspecific competitor. The nonspecific competitor reaction used an oligonucleotide with a different sequence to the specific oligonucleotide. An additional aliquot was prepared and loaded onto the gel, which contained all reagents with the exception of sample (negative control or cold probe). Protein-DNA complexes were separated from the free DNA probe by electrophoresis through 6% native polyacrylamide gels. Gels were dried under vacuum on Whatmann DE-81 paper and exposed to Amersham Hyperfilms at −80°C.

**Western blot analysis.** Lysate proteins were fractionated by SDS-PAGE and Western blotting was performed using the corresponding primary antibodies. Bound antibody was detected by enhanced chemiluminescence. Membrane rehybridization with β-actin antibody was performed for loading accuracy (27).
Results

Cirrhosis and HPS in CBDL rats. Plasma AST, ALT, and ALP activities and bilirubin concentration, portal pressure, and relative liver, lung, and spleen weights did not differ between the untreated sham-operated group and the Sham-Q group. They were significantly higher in untreated CBDL rats than in Sham rats and significantly reduced by quercetin in the CBDL group (Table 1). The AaO₂ was significantly reduced by 72% in CBDL-Q rats compared to the untreated CBDL group, indicating that the flavonoid markedly reduces HPS severity (Table 1).

The untreated CBDL group had evidence of biliary cirrhosis and nodular liver with intense ductular proliferation and fibrotic bridges, whereas cirrhotic rats treated with quercetin had a marked reduction in ductular proliferation (Fig. 1). Lungs from Sham-Q and untreated sham-operated rats had normal architecture of pulmonary parenchyma and vessels of normal diameter. The diameter of pulmonary vessels increased after CBDL and was significantly reduced by treatment with quercetin (Fig. 2).

TBARS, SOD, and NOx in lung. The 80 and 70% increases in TBARS and NOx concentrations, respectively, and the 69% decrease in SOD activity in CBDL rats with cirrhosis compared with sham-operated rats confirmed the presence of oxidative stress in lung tissue associated with cirrhosis-related HPS. These variables were normalized in the CBDL-Q group and they did not differ from those in control rats (Table 2).

Mediators of pulmonary alterations after CBDL. Next, we assessed the effects of quercetin on the pulmonary endothelium (lung ETB-receptor and eNOS) and on pulmonary intravascular macrophages (lung ED1, iNOS, and HO-1) after CBDL. ETB receptor and eNOS mRNA levels were 1.4- and 1.1-fold greater in untreated cirrhotic compared with sham-operated rats (P < 0.05) and were normalized in CBDL-Q rats. Moreover, iNOS and HO-1 mRNA expression in the lungs were 1.5- and 2.4-fold greater and were normalized in CBDL-Q rats (Table 3).

To confirm the accumulation of pulmonary intravascular macrophages in relation to iNOS and HO-1 alterations, we assessed levels of ED1, a specific marker for monocytes/macrophages. The qualitative increase in ED1 was confirmed by Western blotting, which indicated that the level in CBDL rats was 4.6 times that in the Sham group. After quercetin treatment, macrophage accumulation, as assessed by lower ED1 protein levels, was reduced by 54% (Fig. 3).

Pulmonary NF-κB signaling pathway. The signal intensity obtained by EMSA demonstrated that the NF-κB binding activity to NF-κB consensus sequence in lung tissue from untreated CBDL rats was 50% greater than in Sham rats (P < 0.05). Binding activity did not differ from that in sham-operated rats in the CBDL-Q group (Fig. 4).

Progression of cirrhosis. To determine whether quercetin was able to ameliorate cirrhosis, we analyzed the effect on the mRNA expression of genes involved in hepatic fibrogenesis. Collagen type I, procollagen type IV, TGFβ, and α-SMA were 7.4-, 5.3-, 2.9-, and 4.0-fold greater in untreated CBDL rats compared to Sham rats, respectively. Administration of quercetin to cirrhotic rats with established bridging fibrosis resulted in 18–28% lower expression levels compared to untreated CBDL rats (Fig. 5).

ET-1 and HO-1 mRNA after CBDL. To gain further insight into the sequence of molecular events involved in the onset and progression of HSP, we evaluated liver ET-1 and HO-1 mRNA levels, which were 16.7- and 2.4-fold greater in CBDL rats than in Sham rats. Compared to untreated CBDL rats, levels were 33 and 25% lower, respectively, in those treated with quercetin (Table 3).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Sham-Q</th>
<th>CBDL</th>
<th>CBDL-Q</th>
<th>Q</th>
<th>CBDL</th>
<th>Q × CBDL</th>
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<tr>
<td>Liver weight, g/100 g weight</td>
<td>2.9 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>6.1 ± 0.4*</td>
<td>4.4 ± 0.3*</td>
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<td>Lung weight, g/100 g weight</td>
<td>0.43 ± 0.02</td>
<td>0.42 ± 0.02</td>
<td>0.54 ± 0.03*</td>
<td>0.47 ± 0.02*</td>
<td>NS</td>
<td>0.0018</td>
<td>NS</td>
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<td>Spleen weight, g/100 g weight</td>
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<td>0.35 ± 0.03</td>
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<td>0.48 ± 0.04*</td>
<td>NS</td>
<td>0.0001</td>
<td>NS</td>
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<td>Plasma AST, U/L</td>
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<td>66 ± 2</td>
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<td>0.0011</td>
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<td>38 ± 3</td>
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<td>145 ± 9</td>
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<td>Plasma total bilirubin, μmol/L</td>
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<td>4.6 ± 0.3</td>
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<td>75.8 ± 11.8*</td>
<td>0.0011</td>
<td>0.0001</td>
<td>0.0009</td>
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<td>Plasma direct bilirubin, μmol/L</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>107.7 ± 7.4*</td>
<td>49.1 ± 13.9*</td>
<td>0.0014</td>
<td>0.0001</td>
<td>0.0014</td>
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<td>aPVR, mm Hg</td>
<td>11.9 ± 1.6</td>
<td>11.2 ± 0.90</td>
<td>19.7 ± 1.9*</td>
<td>13.0 ± 0.90*</td>
<td>0.0053</td>
<td>0.0011</td>
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<td>PaO₂, mm Hg</td>
<td>90 ± 2</td>
<td>81 ± 4</td>
<td>65 ± 5*</td>
<td>73 ± 2*</td>
<td>NS</td>
<td>0.0071</td>
<td>NS</td>
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<td>AaO₂, mm Hg</td>
<td>5.8 ± 1.2</td>
<td>5.5 ± 1.6</td>
<td>28.8 ± 3.0*</td>
<td>8.2 ± 2.2*</td>
<td>0.0058</td>
<td>0.0014</td>
<td>0.0072</td>
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1 Data are means ± SEM, n = 11. *P < 0.05 vs. Sham rats and Sham-Q rats; $P < 0.05$ vs. CBDL rats. NS, $P ≥ 0.05$.
2 PVP, Portal venous pressure; PaO₂, partial pressure of oxygen.

Statistical analysis. Means and SEM were calculated. Data were analyzed using a 2 (quercetin-treated and quercetin-untreated rats) × 2 (cirrhotic and noncirrhotic rats) ANOVA. Post hoc comparisons were conducted using the Newman-Keuls test. A value of $P < 0.05$ was considered significant. All calculations were performed using the SPSS 14.0 statistical software.
Discussion

Currently, no effective medical therapies for HPS exist and liver transplantation is the only successful treatment (28). Therefore, it is essential to develop novel therapeutic strategies and evaluate agents targeted at likely pathogenetic mechanisms. In the present study, quercetin decreased oxidative damage, nuclear translocation of NF-κB, the expression of lung iNOS, eNOS, HO-1, and ETB-receptor, and the severity of HPS, and also markedly reduced the expression of liver collagen I, procollagen IV, TGFβ,

FIGURE 1  Micrographs of hepatic tissue (original magnification: 100×). (A,D) Sham rats; (B,E) CBDL rats; (C,F) CBDL-Q rats. Tissue samples were counterstained with hematoxylin-eosin (A–C) and Sirius red (D–F). (A,D) Normal liver histology. (B,E) Intense ductular proliferation. (C,F) Marked reduction in ductular proliferation. A color version of this figure is available (Supplemental Fig. 1).

FIGURE 2  Micrographs of pulmonary tissue (original magnification: 200×). Tissue samples were counterstained with hematoxylin-eosin. (A) Sham rats; (B) Sham-Q rats; (C) CBDL rats; (D) CBDL-Q rats. (A,B) Normal architecture of pulmonary parenchyma and vessels of normal diameter. (C) Vessels of increased diameter and (D) showing a marked reduction in the diameter of the pulmonary vessels. A color version of this figure is available (Supplemental Fig. 2).
ET-1 and HO-1, indicating that beneficial effects on the complications of cirrhosis, such as HPS or hypertension portal, could be due to the antioxidant and antifibrotic role of the flavonoid in the liver.

CBDL rats are widely used as a model of cirrhosis and portal hypertension. Although the frequency of HPS differs in humans and CBDL rats, the availability of an animal model to study mechanisms involved in the development of HPS provides considerable insight into potential mechanisms of human disease (20). The cirrhotic rats had impaired arterial oxygenation (11,20), as demonstrated by an increased AaO2. As previously documented using other antioxidant agents (29), the administration of quercetin to CBDL rats normalized the alveolar-arterial oxygen gradient, leading to an improvement of hypoxemia. Several studies found that exhaled NO concentrations were paralleled the increase in pulmonary NO production by ET-1 (32). Accordingly, administration of a selective ETB-receptor antagonist to CBDL rats decreases pulmonary endothelial eNOS and ETB-receptor levels and significantly improves HPS (5). The present study supports the hypothesis that rat pulmonary microvascular ETB-receptor overexpression contributes to enhanced eNOS activation and NO production in response to CBDL. Normalization of ETB-receptor mRNA levels with quercetin administration prevented the progression of HPS and corrected both eNOS messenger RNA induction and pulmonary NO concentrations. Studies using NOS inhibitors have demonstrated that NO overproduction plays a central role in HPS (29,33). Some investigators have shown that expression of eNOS, but not iNOS, is increased in the systemic vascular bed (34). In contrast, others (35) have suggested that iNOS rather than eNOS overexpression causes the NO activity increase seen in pulmonary arteries. Our data are consistent with increased expression of iNOS and, to a lesser extent, eNOS in the lungs of untreated cirrhotic rats, leading to increased pulmonary production of NO, which contributes to the genesis of HPS. In a previous study of cirrhotic rats, it was found that pulmonary NO production increased mainly in relation to iNOS overexpression in macrophages sequestered in pulmonary microvessels and that inhibition by antioxidant agents of NO overproduction prevented HPS (29). In our study, pulmonary intravascular macrophage accumulation also occurred in CBDL rats and was reflected in

### Table 2: TBARS, SOD, and total NOx in lung in Sham and CBDL rats that were or were not treated with quercetin

<table>
<thead>
<tr>
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<th>Sham</th>
<th>Sham-Q</th>
<th>CBDL</th>
<th>CBDL-Q</th>
<th>ANOVA</th>
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<td>TBARS, nmol/mg protein</td>
<td>0.94 ± 0.09</td>
<td>0.71 ± 0.09</td>
<td>1.72 ± 0.03</td>
<td>0.80 ± 0.02</td>
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<td>SOD, U/mg protein</td>
<td>8.0 ± 0.5</td>
<td>6.9 ± 0.7</td>
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<td>NOx, mmol/L</td>
<td>86 ± 7</td>
<td>95 ± 6</td>
<td>147 ± 10</td>
<td>105 ± 7</td>
<td>NS</td>
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1 Data are means ± SEM, n = 11. *P < 0.05 vs. Sham rats and Sham-Q rats; **P < 0.05 vs. CBDL rats. NS, P ≥ 0.05.

### Table 3: Levels of mRNA of ETB-receptor, eNOS, iNOS, and HO-1 in lung and levels of mRNA of collagen type I, procollagen type IV, TGFβ, α-SMA, ET-1, and HO-1 in liver after CBDL and treatment with quercetin

<table>
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<th>% of Sham</th>
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<tr>
<td>ETB-receptor, %</td>
<td>100 ± 6</td>
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<tr>
<td>eNOS</td>
<td>100 ± 10</td>
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<td>iNOS</td>
<td>100 ± 12</td>
<td>115 ± 28</td>
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<td>HO-1</td>
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Liver

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<tr>
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<th>Collagen type I</th>
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<th>TGFβ</th>
<th>α-SMA</th>
<th>ET-1</th>
<th>HO-1</th>
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<tr>
<td>% of Sham</td>
<td>133 ± 21</td>
<td>110 ± 15</td>
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<td>128 ± 9</td>
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<td></td>
<td>839 ± 32</td>
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<td>503 ± 23</td>
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<td></td>
<td>602 ± 28</td>
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<td>ANOVA</td>
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<td>0.0001</td>
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1 Values are means ± SEM, n = 11 (duplicate samples from 3 separate experiments for each group). *P < 0.05 vs. Sham rats and Sham-Q rats; **P < 0.05 vs. CBDL rats.
2 Levels of mRNA were normalized to HPRT.
increased ED1 levels; quercetin treatment decreased intravascular macrophage recruitment compared to untreated cirrhotic rats.

As experimental HPS progresses, there is a steady accumulation of intravascular macrophages. These cells transiently produce iNOS (11,29) and progressively produce HO-1 (4,11). These events contribute to further vasodilatation through production of iNOS-derived NO and HO-1–derived CO. There is solid evidence that crosstalk between the NOS and HO systems occur. Our findings are in concordance with those suggesting that NO derived from iNOS and, potentially, eNOS, are significantly associated with increased HO-1 expression in lung (11). Increased expression of HO-1 also has been associated with treatment with quercetin in vitro studies (36). However, in the present study, HO-1 expression did not differ between Sham and Sham-Q rats, perhaps due to quercetin’s limited bioavailability and metabolism, which make it difficult to extrapolate molecular mechanisms identified by in vitro approaches to in vivo situations. Overexpression of the isoform HO-1 was found only in the untreated CBDL group. Considering that HO-1 has a protective role against the effects of increased oxidative stress on cell function (37), we speculate that HO-1 may be upregulated in cirrhosis as a homeostatic mechanism against excessive oxidative stress. Thus, the decrease in oxidative stress after treatment with quercetin in cirrhotic rats, represented by the restoration of plasma TBARS, NO, and SOD, may be related to the normal levels of HO-1 mRNA in these rats. A second possibility is suggested by data showing that NO inhibition can block the increase in pulmonary HO-1 after CBDL (38). Taken together, both explanations could be related to the potential inactivation of the redox-sensitive transcription factors that regulates the expression of these enzymes.

One of the most important signaling pathways that could be activated during HPS is NF-κB, because this factor is activated by a variety of external stimulants, including reactive oxygen species, cytokines, and vascular shear stress. Once NF-κB is activated, it migrates to the nucleus, resulting in changes in the expression of different enzymes, including NOS and HO-1 (9,10). This study demonstrated that mRNA levels of iNOS, eNOS, HO-1, and ETB-receptor increased in parallel to NF-κB activation in lung from untreated CBDL rats. Moreover, mRNA levels of the above-cited genes were not affected in cirrhotic rats treated with the flavonoid. Quercetin administered after the onset of hepatic injury significantly attenuated the development of HPS, abolishing NF-κB activation and downregulating pulmonary ETB-receptor and iNOS, eNOS, and HO-1 mRNA levels.

Histological findings demonstrated that the untreated CBDL group had evidence of biliary cirrhosis. Excessive accumulation of fibrillar collagens I, III, and IV leads to the production of a wide set of profibrogenic molecules that rapidly accelerate development of fibrosis (39). Collagen I and procollagen IV gene
expression were considerably lower in CBDL-Q rats compared to CBDL rats, effects which could be explained by quercetin’s protective effect against oxidative stress damage, which prevented scarring. Consistent with this, TGFβ, the most potent inductor of fibrosis, was lowered by quercetin treatment, confirming its antifibrotic role (13). These effects could be linked to an inhibitory effect of quercetin on TGFβ-induced expression of matrix genes by hepatic stellate cells and can also be explained by a reduction in hepatic stellate cell proliferation/differentiation as demonstrated by decreased mRNA levels of α-SMA and immunostaining of α-SMA-positive cells. Moreover, quercetin had a hepatic-protective effect, as indicated by improvement in markers of hepatic damage, including ALT, AST, ALP, and bilirubin concentrations in cirrhotic rats treated with the flavonoid.

Our data also demonstrated that hepatic injury after CBDL resulted in enhanced hepatic ET-1 and HO-1 mRNA levels, which are associated with the development of molecular and functional alterations in HPS. The improvement in damaged liver in CBDL-Q rats closely paralleled the decrease in ET-1 and HO-1 mRNA levels in the liver. Thus, quercetin was particularly effective in decreasing gene expression of hepatic mediators involved in HPS after CBDL, suggesting that effects of this antioxidant on this syndrome are related to the prevention of the evolution of cirrhosis.

In conclusion, the present findings have potentially important clinical and mechanistic implications for treating and understanding HPS, suggesting that the protective effect of quercetin could be due to a combination of different mechanisms on various cell types. First, our results show that in the CBDL rat model, quercetin administered after the onset of hepatic injury significantly improved HPS by reducing oxidative stress, abolishing NF-κB activation and downregulating pulmonary ETB-receptor and iNOS, eNOS, and HO-1 mRNA levels. Second, from a practical standpoint, we have shown that quercetin treatment, initiated following the establishment of liver injury in CBDL rats, decreased the severity of the subsequent HPS and that the positive effect on limiting cirrhotic evolution contributed to this effect.

### Literature Cited


