Chronic administration of ursodeoxycholic acid decreases portal pressure in rats with biliary cirrhosis

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ABSTRACT

Liver cirrhosis is characterized by increased IHR (intrahepatic resistance) and lipid peroxidation, and decreased antioxidative defence. The present study investigates the effects of administration for 1 month of the antioxidant UDCA (ursodeoxycholic acid) in BDL (bile-duct-ligated) cirrhotic rats. Splanchnic haemodynamics, IHR, hepatic levels of TBARS (thiobarbituric acid-reacting substances), GSH (glutathione), SOD (superoxide dismutase) activity, nitrite, PIIINP (N-terminal propeptide of type III procollagen) and collagen deposition, histological examination of liver, mRNA expression of PIIIP-α1 (type III procollagen) and TGF-β1 (transforming growth factor-β1), protein expression of TXS (thromboxane synthase) and iNOS (inducible NO synthase), and TXA2 (thromboxane A2) production in liver perfusates were measured. The results showed that portal pressure and IHR, hepatic levels of PIIINP, hepatic collagen deposition, mRNA expression of PIIIP-α1 and TGF-β1, protein expression of iNOS and TXS, and production of TXA2 in liver perfusates were significantly decreased in UDCA-treated BDL rats. The increased levels of hepatic GSH and SOD activity and decreased levels of TBARS and nitrite were also observed in UDCA-treated BDL rats. In UDCA-treated BDL rats, the reduction in portal pressure resulted from a decrease in IHR, which mostly acted through the suppression of hepatic TXA2 production and lipid peroxidation, and an increase in antioxidative defence, leading to the prevention of hepatic fibrosis.

INTRODUCTION

An increase in IHR (intrahepatic resistance), due to fibrosis, scar and nodule formation of liver parenchyma, is an initiator of all of the haemodynamic derangements in cirrhosis [1]. A number of studies have demonstrated that there are contractile elements in cirrhotic livers that are able to constrict, in a reversible and graded manner, in response to various vasoactive substances, such as thromboxanes and products of lipid peroxidation [2–4]. Thus

Key words: antioxidant, cirrhosis, fibrosis, intrahepatic resistance, portal venous pressure, ursodeoxycholic acid (UDCA).

Abbreviations: ALKP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDL, bile-duct-ligated; BDL-V rat, vehicle-treated BDL rat; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; GGT, γ-glutamyl transpeptidase; GSH, glutathione; IHR, intrahepatic resistance; iNOS, inducible NO synthase; LPS, lipopolysaccharide; MAP, mean arterial pressure; PBC, primary biliary cirrhosis; PIIIP, type III procollagen; PIIINP, N-terminal propeptide of type III procollagen; PPP, portal perfusion pressure; PSC, primary sclerosing cholangitis; PVP, portal venous pressure; ROS, reactive oxygen species; RT–PCR, reverse transcription–PCR; Sham-V, sham-operated rats receiving vehicle; SMA, superior mesentery artery; QSMABlood flow; SOD, superoxide dismutase; TB, total bilirubin; TBARS, thiobarbituric acid-reacting substances; TGF-β1, transforming growth factor-β1; TXA2, thromboxane A2; TXB2, thromboxane B2; TXS, thromboxane synthase; UDCA, ursodeoxycholic acid; BDL-UDCA rat, UDCA-treated BDL rat.

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inhibition of the ongoing fibrosis and modulation of these dynamic components to decrease IHR has become a key therapeutic strategy in cirrhosis [5].

The importance of increased production of hepatic thromboxanes in increased IHR in cirrhosis had been established [2]. On the other hand, an increase in lipid peroxidation and a decrease in antioxidative defence had been reported in human and animals with cirrhosis [4,6]. Oxidative stress can be defined as a loss of balance between the products of lipid peroxidation and antioxidants [7]. This imbalance leads to the increased oxidation of macromolecules, including proteins, lipids and carbohydrates, and the release of ROS (reactive oxygen species) [8]. In cirrhotic livers, increased oxidative stress and production of ROS have been found to accelerate the fibrogenic process [6,9,10].

In cirrhosis, the bacterial endotoxin [LPS (lipopolysaccharide)] generated by normal intestinal flora gains access to the systemic circulation as a result of its impaired hepatic elimination and the increase in porto-systemic shunting [11]. The production of NO via iNOS (inducible NO synthase) is prominent in the liver after stimulation with endotoxin (LPS) [12,13]. Previous studies have shown that iNOS-generated NO not only directly contributes to hepatotoxicity, but that it also up-regulates the inflammatory response [12,13]. iNOS-generated NO can bind irreversibly to multiple components of the mitochondrial respiratory chain, affecting cell respiration and precipitating necrosis and fibrosis [8,13].

Accumulation of hydrophobic bile acids in cholestatic rat livers has been found to release ROS and increase the pro-inflammatory cytokines in rat livers [11,13]. Biliary cirrhosis is a well-established animal model with cholestasis and markedly increased ROS [4,14]. UDCA (ursodeoxycholic acid) is a stereoisomer of chenodeoxycholic acid but is relatively more hydrophilic in nature [15]. UDCA has been found to inhibit the solubilization of cholesterol and phospholipids in cell membranes by hydrophobic bile salts and to protect against bile salt toxicity in hepatocytes [16,17].

UDCA has been shown to be beneficial in the treatment of patients with liver diseases, such as PBC (primary biliary cirrhosis), and liver injury in rats [16,18]. Some peripheral effects of chronic UDCA treatment have been reported in cirrhosis [18–20]; however, the effects of UDCA in cirrhotic livers are still unclear. Therefore the aim of the present study was to investigate the effects of chronic UDCA administration in the livers of rats with biliary cirrhosis.

MATERIALS AND METHODS

Animals
Adult male Sprague–Dawley rats (250–350 g) were used in all of the experiments. Cirrhosis with portal hypertension was produced by bile-duct ligation, as described previously [4]. All rats were caged at 24 °C, with a 12-h light/dark cycle, and allowed free access to food and water. Animal studies were approved by the Animal Experiment Committee of the University and conducted according to the ‘Guides for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science, U.S.A. Immediately after surgery, BDL (bile-duct ligated) rats were divided into two groups to receive vehicle (BDL-V) or UDCA (BDL-UDCA; 50 mg·kg−1 of body weight·day−1) respectively, for 30 consecutive days according to a previous study [21]. Drugs were given by oral gavage. One group of sham-operated rats receiving vehicle (Sham-V) was used for comparison.

Chemicals
GSH (glutathione)-400 colorimetric assay, SOD (superoxide dismutase) activity assay, TBARS (thiobarbituric acid-reacting substances) assay and TXB2 (thromboxane B2) ELISA kits, and the monoclonal antibody for TXS (thromboxane synthase) and iNOS were purchased from Cayman Chemicals. The intact PIIIP (type III procollagen) RIA kit was purchased from Orion Diagnostica. PCR primers for TGF-β1 (transforming growth factor-β1) and G3PDH (glyceraldehyde-3-phosphate dehydrogenase), and related substances, were prepared by a DNA synthesizer (Protech Technology). Primers of quantitative real-time RT–PCR (reverse transcription–PCR) for PIIIP-α1 (Rn01437683_m1) and G3PDH (Rn99999916_s1) were purchased from Applied Biosystems. Substances, other than those described above, were purchased from Sigma.

Experiment 1: haemodynamic and liver function measurements
One group of BDL-V rats, one group of BDL-UDCA rats and one group of Sham-V rats were included in this experiment (n = 8 in each study group). All rats were starved 18 h before haemodynamic studies and free access to water. Then all rats were anaesthetized with 100 mg of ketamine/kg of body weight. The femoral artery catheter was inserted to monitor MAP (mean arterial pressure) and to withdraw blood samples. The ileocolic vein was cannulated with PE-10 tubing for measurement of PVP (portal venous pressure). PVP was monitored using a polygraph (Gould) via strain-gauge transducers (Viggo-Spectramed). Meanwhile, a flow probe was placed around the SMA (superior mesentery artery) and connected to a flowmeter (Transonic) for continuous monitoring of Qsma (SMA blood flow) [22]. After haemodynamic measurements had been recorded, the blood samples were collected. The serum activities of AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALKP (alkaline phosphatase), GGT (γ-glutamyl transpeptidase) and TB (total
Experiment 2: liver perfusion study

Another one group of BDL-V rats, one group of BDL-UDCA rats and one group of Sham-V rats were included in this experiment (n = 8 in each group). Isolated cirrhotic and normal livers were used for the in situ recirculating perfusion study, as described previously with a constant flow of 30 ml/min [23]. A total of 6 ml of perfusates were obtained before (0-min time point) and 30 min (30-min time point) after the recirculating system was collected to estimate the basal production of TXB2. TXB2 is the stable and inactive metabolite of TXA2 (thromboxane A2). The production of TXB2 was expressed as pg·ml⁻¹·min⁻¹·g⁻¹ of liver weight. TXB2 production was quantified in duplicate using a commercially available ELISA kit. The global viability of each liver was quantified in duplicate using a commercially available ELISA kit. The quality of RNA was verified using the Tripure reagent, according to the manufacturer’s instructions. Furthermore, total RNA was isolated from homogenized livers using the Tripure reagent, according to the manufacturer’s instructions. The quality of RNA was verified by ethidium bromide staining of the rRNA bands on an agarose gel. PHIIIP-α1 mRNA was measured by quantitative real-time RT-PCR using an ABI Prism 7000 Sequence Detection System (Applied Biosystems), according to the manufacturer’s protocol. Predeveloped TaqMan assay reagent for 18S rRNA was used as an endogenous control.

Hepatic TGF-β1 mRNA was also assessed by semi-quantitative RT-PCR. First-strand cDNA was prepared from 1 μg of deoxyribonuclease-treated total RNA using oligo(dT)₁₂₋₁₈ primers and the superscript amplification system. The target sequence (TGF-β1) and endogenous standard sequence G3PDH were amplified simultaneously from either 1.25 or 2.5 μl of first-strand cDNA for TGF-β1 and G3PDH respectively, in 25 μl reaction mixtures. Each reaction contained 10 mmol/l Tris/HCl (pH 8.3), 1.5 mmol/l MgCl₂, 50 mmol/l KCl, 0.2 mmol/l DTP, 1 unit of Taq DNA polymerase and 0.4 μmol/l of specific primer: TGF-β1, 5’-TATAGCAACATCCTGGCG-3’ (sense) and 5’-TGCTTGTCAGGAGCA-TGTG-3’ (antisense); G3PDH, 5’-TCTCAAGATTGT-CAGCAA-3’ (sense) and 5’-AGATCCACAACGGATACATT-3’ (antisense). The following thermal cycle was used: denaturation at 94°C for 5 min, three temperature cycles at 94°C for 30 s, 58°C for 30 s and 72°C for 30 s, followed by a final extension step at 72°C for 7 min using a GeneAmp PCR system 2400 DNA thermal Cycler (PerkinElmer). The number of cycles was optimized at 30 after examination of the yield of PCR products at a range of 10–40 cycles. The amplified PCR products were electrophoresed at 75 V through a 2% (w/v) agarose gel for 1 h. The PCR products were size-fractionated on agarose gels and visualized by ethidium bromide oxidation, were determined with a commercially available ELISA kit, according to the manufacturer’s instructions. Finally, the hepatic nitrite concentrations were estimated by the Griess reaction, as described previously [24]. Nitrite is the stable metabolite of NO. Hepatic nitrite concentrations are expressed as μmol·l⁻¹·g⁻¹ of protein. In addition, hepatic collagen deposition was also measured, as described previously [23].

Experiment 4: hepatic level of intact PIIINP, and mRNA levels of hepatic PHIIIP-α₁ and TGF-β₁

Liver samples were obtained from the groups of rats in Experiment 2. A previous study has shown that the hepatic levels of PIIINP reflect the activity of the fibrogenic process, rather than the cumulative fibrotic state, which was assessed histological [25]. A commercially available research kit containing ¹²⁵I-labelled PIIINP and the corresponding specific antibody from rabbit was used. All tests were performed in duplicate and a standard was run with all determinations for quality control. Furthermore, total RNA was isolated from homogenized livers using the TriPure reagent, according to the manufacturer’s instructions. The quality of RNA was verified by ethidium bromide staining of the rRNA bands on an agarose gel. PHIIIP-α1 mRNA was assessed by quantitative real-time RT-PCR using an ABI Prism 7000 Sequence Detection System (Applied Biosystems), according to the manufacturer’s protocol. Predeveloped TaqMan assay reagent for 18S rRNA was used as an endogenous control.

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staining. The mRNA transcripts were photographed using the Pharmacia Biotech System and scanned using Imaging Master. Relative expression of the target gene mRNA (PIIIP-γ, TGF-β1) was normalized to the amount of G3PDH mRNA in an identical cDNA sample using the standard curve method recommended by the manufacturer.

**Experiment 5: protein expression of hepatic TXS and iNOS**
Liver samples were obtained from each rat in the groups in Experiment 2. Protein expression of hepatic TXS and iNOS was then measured as described previously [23].

**Experiment 6: histological examination**
Liver samples were obtained from each rat in the groups in Experiment 2. The liver tissue was fixed in 10% (v/v) formalin and then embedded in paraffin, cut into 5-μm-thick sections, stained with Masson’s Trichrome and examined randomly under light microscopy by an experienced pathologist.

**Statistical analysis**
Statistical analysis was performed using the SPSS 11.0 statistical package. All results were expressed as means ± S.D. Comparisons within each group were performed using Student’s t test for paired and unpaired data, and comparisons between groups were by ANOVA, followed by the Student–Newman–Keuls multiple range test. The Wilcoxon test was used when appropriate. To compare mRNA levels of the different hepatic genes (TGF-β1 and PIIIP-γ), Mann–Whitney tests were used. Results were considered statistically significant at a P value of <0.05.

**RESULTS**

**Effect of UCDA on haemodynamic and liver function**
All cirrhotic rats had portal hypertension, ascites and splenomegaly upon gross inspection. Serum levels of AST, ALT, ALKP, GGT and TB, which reflect acute and chronic hepatobiliary injury, were significantly elevated in BDL-V rats compared with Sham-V rats (Table 1). Body weight was not different between BDL-V and Sham-V rats (results not shown). Liver weight, PVP and QSMA were greater in BDL-V rats than in Sham-V rats (Table 2). PVP, liver weight, and serum levels of AST, ALT, ALKP, GGT and TB in BDL-UDCA rats were significantly lower than in BDL-V rats. QSMA was not different between BDL-V and BDL-UDCA rats, and MAP was also not different between BDL-V and BDL-UDCA rats (93 ± 6 compared with 97 ± 5 mmHg respectively). Mortality was approx. 13% and did not differ between BDL-V and BDL-UDCA rats.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Biochemical results in the experimental groups</th>
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<tr>
<td>Variable</td>
<td>Sham-V</td>
</tr>
<tr>
<td>AST (units/l)</td>
<td>88 ± 10</td>
</tr>
<tr>
<td>ALT (units/l)</td>
<td>54 ± 5</td>
</tr>
<tr>
<td>ALKP (units/l)</td>
<td>216 ± 31</td>
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<tr>
<td>GGT (units/l)</td>
<td>14 ± 2</td>
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<tr>
<td>TB (mg/l)</td>
<td>0.6 ± 0.03</td>
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<tr>
<td>Hepatic TXB2 production</td>
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<th>Table 2</th>
<th>Haemodynamic results in the experimental groups</th>
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<tr>
<td>Variable</td>
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<tr>
<td>PVP (mmHg)</td>
<td>8.1 ± 1.1</td>
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<tr>
<td>QSMA (ml/min)</td>
<td>8.6 ± 3.2</td>
</tr>
<tr>
<td>Wliver (g)</td>
<td>26.3 ± 4.3</td>
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<tr>
<td>PPP (mmHg)</td>
<td>9.1 ± 1.7</td>
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<tr>
<td>IHR (mmHg · ml−1 · min−1 · g−1 of Wliver)</td>
<td>7.9 ± 4.1</td>
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<tr>
<td>Hepatic collagen deposition</td>
<td>7.1 ± 2.1</td>
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**Effect of UCDA on liver perfusion**
PPP and the calculated IHR were significantly higher in BDL-V rats compared with Sham-V rats (Table 2). In addition, lower PPP and IHR were observed in BDL-UDCA rats compared with BDL-V rats (Table 2). A significant decrease in the production of TXB2 in liver perfusates was found in cirrhotic livers of BDL-UDCA rats compared with BDL-V rats (Table 1).

**Effect of UCDA on the hepatic GSH, SOD activity, products of lipid peroxidation, nitrite and hepatic collagen deposition**
Lower hepatic GSH level and SOD activity (Figure 1), higher hepatic levels of TBARS and nitrite (Figure 2), and higher hepatic collagen deposition (Table 2) were observed in BDL-V rats compared with Sham-V rats. In BDL rats treated for 1 month with UDCA, hepatic GSH level and SOD activity were significantly increased compared with BDL-V rats (Figure 1). Hepatic levels of TBARS, nitrite and hepatic collagen deposition were significantly decreased by 1 month of treatment with UDCA compared with BDL-V rats (Figure 2 and Table 2).
UDCA decreases portal pressure in rats with biliary cirrhosis

Effect of UDCA on the hepatic level of intact PIIINP and PIIIP-α1 mRNA

In BDL-V rats, the hepatic levels of PIIINP (Figure 3A) and PIIIP-α1 mRNA (Figure 3B) were significantly higher compared with Sham-V rats. Treatment of BDL rats for 1 month with UDCA significantly decreased the hepatic level of PIIINP and corresponding hepatic PIIIP-α1 mRNA compared with BDL-V rats (Figure 3). In livers from BDL-V rats, hepatic TGF-β1 mRNA was significantly higher than in livers from Sham-V rats (Figure 4). Additionally, treatment of BDL rats for 1 month with UDCA significantly inhibited the level of hepatic TGF-β1 mRNA in the livers of these rats compared with BDL-V rats (Figure 4).

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Figure 5  Expression of hepatic TXS (A) and iNOS (B) in the experimental groups
Top panels, representative Western blots of hepatic TXS and iNOS. Bottom panels, densitometric analysis of the Western blots for hepatic TXS and iNOS compared with the control protein G3PDH. a P < 0.01 compared with Sham-V rats; b P < 0.01 compared with BDL-V rats.

Effect of UCDA on the protein expression of hepatic TXS and iNOS in BDL rats
Proteins expression of hepatic TXS and iNOS were higher in BDL-V rats than in Sham-V rats. Furthermore, lower protein expression of hepatic TXS and iNOS were observed in BDL-UDCA rats compared with BDL-V rats (Figure 5).

Effect of UCDA on liver histology in BDL rats
Compared with livers from BDL-V rats, treatment with UCDA for 1 month significantly decreased collagen accumulation in livers from BDL-UDCA rats (Figure 6).

DISCUSSION
The results of the present study show, interestingly, that chronic administration of UDCA significantly decreased PVP in BDL rats without altering Q:\textsubscript{SM\textsubscript{A}}. These results indicate that the decrease in PVP resulted mainly from a decrease in IHR. In fact, in the present study, we observed a significant decrease in IHR in BDL-UCDA rats. In cirrhosis, increased IHR is, at least partly, caused by an increase in vasoactive substances, such as TXA\textsubscript{2}, due to chronic increased hepatic lipid peroxidation and hepatic inflammation.

In the present study, hepatic TXA\textsubscript{2} production was also significantly inhibited in BDL-UCDA rats. TXA\textsubscript{2} can act as a vasoconstrictor in the hepatic microcirculation [23], thus the decrease in IHR in BDL-UDCA rats resulted,
at least partly, from the inhibition of hepatic TXA$_2$ release from the liver.

UDCA was originally isolated from the Chinese medicine Yutan, which is derived from the bile of adult Chinese black bears. UDCA can increase GST (glutathione transferase) activities in rat livers [16,18], and previous studies in rats with biliary cirrhosis have shown that chronic administration of UDCA can prevent the depletion of hepatic GSH [26,27]. In addition, chronic administration of UDCA has been found to increase SOD activity in experimental alcoholic steatohepatitis [28]. Consistent with these findings, in the present study, a significant increase in hepatic GSH level and SOD activity were observed in livers from BDL-UDCA rats.

It is well-established that the elevation of serum bilirubin and liver function are a result of chronic hepatic inflammation. In the present study, the serum levels of liver enzymes and bilirubin were significantly decreased by UDCA treatment in cirrhotic rats, indicating an amelioration of hepatic inflammation. High plasma levels of products of lipid peroxidation, which can damage an array of biomolecules in liver tissue, have been observed in patients and rats with cholestasis [15,17,18,29,30]. In the present study, higher hepatic levels of TBARS were observed in cirrhotic rats, but treatment for 1 month with UDCA significantly suppressed this increase. Furthermore, the inhibition of hepatic inflammation was associated with the inhibition of hepatic lipid peroxidation in BDL-UDCA rats. Thus it is reasonable to suggest that the UDCA-related inhibition of hepatic lipid peroxidation led to the improvement in hepatic inflammation in BDL-UDCA rats.

The role of NO in liver damage remains controversial [31,32]. Some studies have reported a protective role of NO in liver injury [33,34], whereas other have suggested that excessive NO production by iNOS may cause hepatic injury [35,36]. Consistent with a previous study [37], we found in the present study higher hepatic nitrite levels and hepatic iNOS protein expression in livers of BDL-V rats compared with livers of Sham-V rats. We have also shown in the present study that treatment of BDL rats for 1 month with UDCA significantly inhibited hepatic iNOS expression and the corresponding hepatic levels of nitrite. In VSMCs (vascular smooth muscle cells) and a mouse macrophage cell line, administration of UDCA was found to inhibit the activity of iNOS and the LPS-induced release of NO [24,38,39]. A previous study has shown that inhibition of hepatic iNOS expression can attenuate damage in livers of BDL rats [39]. In the present study, the inhibition of hepatic iNOS-related nitrite release was accompanied by the amelioration of hepatic inflammation in BDL-UDCA rats. Thus it is possible that the UDCA-related inhibition of iNOS activity plays, at least in part, a role in the mechanism of ameliorating hepatic inflammation in BDL-UDCA rats.

UDCA is mainly metabolized in the liver and, in fact, the effects of chronic UDCA administration on the hepatic microcirculation in cirrhosis are still unclear. It has been observed that large amounts of hydrophobic bile acids accumulate in animals with cholestasis. Hydrophobic bile acids are known to induce hepatocyte damage because of their detergent properties via the disruption of cholesterol-rich cellular membranes [17]. It has been suggested that hepatic retention of endogenous hydrophobic bile acids can stimulate the generation of ROS, oxidative injury and hepatocyte apoptosis in cirrhotic animals [14,16]. A major hepatic effect of UDCA is to replace hydrophobic bile acids with hydrophilic bile acids [40,41]. Therefore administration of UDCA might prevent the deleterious effects caused by the accumulation of hydrophobic bile acids and inhibit oxidative injury in cirrhotic livers. These previous observations support further the anti-inflammatory effect of UDCA in BDL-UDCA rats found in the present study.

It is well-established that increased IHR in cirrhosis is mainly caused by structural changes during the process of hepatic fibrosis and cirrhosis. The dynamic component, which is modulated by various vasoactive substances, only partly regulates IHR in cirrhosis [3]. Furthermore, it is well known that increased oxidative stress and hepatic inflammation may initiate hepatic fibrosis and cirrhosis [4,9,14,29]. Thus administration of UDCA might have some benefits in the prevention of liver fibrosis. Indeed, in patients with PBC, chronic UDCA treatment has been shown to delay disease progression by preventing the process of fibrosis [42]. Enhanced TGF-β$_1$ expression and increased hepatic collagen deposition have been reported in experimental biliary cirrhosis and humans with biliary cirrhosis [43,44]. Chronic administration of UDCA has been found to suppress the serum level of TGF-β$_1$ in patients with PBC [45]. Further studies have shown that UDCA inhibited the TGF-β$_1$-induced apoptosis in rat hepatocytes [46]. In the present study, hepatic TGF-β$_1$ and collagen deposition served as fibrogenic markers [42]. Additionally, histological examination of cirrhotic livers also revealed a decrease in hepatic collagen deposition in BDL-UDCA rats. Accordingly, a decrease in liver weight in BDL-UDCA rats was observed in the present study. In addition, the prevention of hepatic fibrosis was associated with the UDCA-related inhibition of hepatic inflammation and oxidative stress in BDL-UDCA rats. These results support further the roles of hepatic oxidative stress and hepatic inflammation in the initiation and maintenance of hepatic fibrosis in cirrhosis.

UDCA is a physiological component of normal human bile and has been used to treat various cholestatic liver diseases including PBC and PSC (primary sclerosing cholangitis). A wide range of doses of UDCA (13–25 mg · kg$^{-1}$ · day$^{-1}$) of body weight · day$^{-1}$) have been used to treat patients with PBC with beneficial effects [47]. Additionally, UDCA (10–30 mg · kg$^{-1}$ of body weight · day$^{-1}$)
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has been found to improve liver biochemistries in patients with PSC [48,49]. A recent study has also reported that a high dose of UDCA (30 mg · kg⁻¹ of body weight · day⁻¹) is well-tolerated and is associated with an improvement in survival probability of patients with PSC [50]. Tasci et al. [21] have reported that a high dose of UDCA (30 mg · kg⁻¹ of body weight · day⁻¹) can regress hepatic fibrosis in cirrhotic rats. Thus that dose of UDCA (50 mg · kg⁻¹ of body weight · day⁻¹) was chosen in the present study to investigate the chronic effect of UDCA on hepatic microcirculation in cirrhotic rats.

In the present study, both mesenteric blood flow and MAP were unchanged in BDL-UDCA rats. These results indicate that systemic vasodilation in cirrhotic rats was not modified by treatment for 1 month with UDCA. In fact, studies on the effects of chronic administration of UDCA on systemic haemodynamics are still limited. In healthy volunteers, 4 weeks of UDCA (750 mg/day) treatment induced a slight decrease in MAP [51]; however, MAP, heart rate, cardiac output and systemic vascular resistance were not changed by treatment for 1 month with UDCA (15 mg · kg⁻¹ of body weight · day⁻¹) in patients with cirrhosis and refractory ascites [20]. In our present study, treatment for 1 month with UDCA (50 mg · kg⁻¹ of body weight · day⁻¹) also did not change mesenteric blood flow and MAP in BDL rats. However, a previous study has shown that biliary enrichment of UDCA increases with increasing dose [48]. Thus in future studies it will be necessary to evaluate the systemic effects of higher doses of UDCA in cirrhosis.

Cumulative evidence suggests that systemic overproduction of NO contributes to systemic vasodilation in cirrhosis [52]. Wei et al. [39] have reported that chronic administration of a specific inhibitor of iNOS, aminoguanidine, decreased systemic NO levels, as well as suppressed iNOS expression and activity in aorta, and improved liver function in BDL rats [39]. A previous study has found that UDCA inhibits the release of iNOS-related NO in aortic smooth muscle cells [24]. Accordingly, a combination of UDCA and a low dose of an iNOS inhibitor, such as aminoguanidine, could have a dramatic effect on systemic vasodilation in cirrhotic rats. However, this hypothesis needs to be explored in future studies.

Taken together, the results of the present study show that the decrease in PVP resulted mainly from a reduction in IHR in BDL-UDCA rats. The UDCA-related decrease in IHR was due to an increase in antioxidative defence, inhibition of hepatic inflammation, suppression of hepatic TXA₂ production and prevention of liver fibrosis in BDL rats.

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