Heme oxygenase/carbon monoxide pathway inhibition plays a role in ameliorating fibrosis following splenectomy

QIU-MING WANG¹, ZHI-JUN DUAN¹, JIAN-LING DU², SHI-BIN GUO¹, XIAO-YU SUN¹ and ZHEN LIU¹

Departments of ¹Gastroenterology and ²Endocrinology, The First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning 116011, P.R. China

Received December 21, 2012; Accepted February 22, 2013

DOI: 10.3892/ijmm.2013.1309

Abstract. Splenectomy is a recognized therapy for liver cirrhosis with splenomegaly, since it decreases free iron concentration that accompanies the destruction of red blood cells. Heme oxygenase (HO)-1 and its by-products, iron and carbon monoxide (CO), play crucial roles in hepatic fibrosis. The aim of the present study was to determine whether splenectomy in cirrhotic rats induced by bile duct ligation (BDL), through the HO/CO pathway, could slow down the development of liver fibrosis. Male Sprague-Dawley rats were divided randomly into the sham, BDL, splenectomy, Fe, zinc protoporphyrin (ZnPP) and cobalt protoporphyrin (CoPP) treatment groups, for inhibiting and inducing HO-1 expression. The level of HO-1 was detected by western blot analysis and reverse transcription-polymerase chain reaction. Serum carboxyhemoglobin (COHb), iron and portal vein pressure (PVP) were also quantified. Liver iron was measured by atomic absorption spectrometry with acetylene-air flame atomization. HO-1 and α-smooth muscle actin (α-SMA) were localized by immunohistochemistry. Liver and spleen iron were visualized by Perls’ Prussian blue staining. Hepatic fibrosis was assessed using hematoxylin and eosin (H&E) staining. Enzyme-linked immunosorbent assay (ELISA) was used to detect serum transforming growth factor-β1 (TGF-β1). The results showed that liver, spleen and serum levels of HO-1, COHb and iron were greatly enhanced in the BDL group compared with the sham group; they were reduced following splenectomy and ZnPP treatment, but were elevated in the CoPP and Fe groups. Hydroxyproline, TGF-β1, α-SMA, PVP and malonaldehyde levels were lower in the splenectomy and ZnPP groups compared to BDL, while higher levels were observed in the CoPP and Fe-treated groups. Our study shows that splenectomy reduces iron and CO levels in part by reducing HO-1 expression, and it decreases portal pressure and slightly decreases hepatic fibroproliferation.

Introduction

The spleen is connected to the other abdominal organs by the portal system. Portal hypertension and splenomegaly are often found in end-stage hepatic cirrhosis. As a treatment option, splenectomy is more widely accepted for patients without a donor suitable for liver transplantation (1). It is reported that splenectomy has beneficial effects on ameliorating reperfusion injury (2,3) and that it also induces a significant reduction in portal system pressure, improving hepatic function (4,5). Splenectomy also improves hepatic microcirculation, decreases spleen-derived endothelin-1, activates eNOS signaling, and inhibits the Rho-kinase pathway in rats with secondary biliary cirrhosis (6). Splenectomy also ameliorates liver injury in rats by mediating heme oxygenase-1 (HO-1) induction, thus yielding beneficial effects of massive hepatectomy and ischemia/reperfusion (7,8). The mechanism by which splenectomy influences cirrhotic liver remains unclear.

Previous studies, including our own, show that the HO/carbon monoxide (CO) pathway is involved in hepatic fibrosis and contributes to the hyperdynamic circulatory syndrome (9,10). HO-1 catalyses the oxidative degradation of heme to CO, free iron, and biliverdin (11). CO, a gaseous messenger similar to NO, leads to the production of cGMP, which mediates vasodilation (12). Our previous studies indicated that the HO/CO pathway may contribute to renal and pulmonary vasodilation in cirrhotic rats induced by bile duct ligation (BDL) (13,14). Whether splenectomy portal vein pressure (PVP) decreases are associated with a change in the HO/CO pathway is not clear.

Cirrhotic patients often have hypersplenism due to splenomegaly. Therefore, leukopenia, thrombocytopenia, and anemia are major concerns in cirrhotic patients (15). Damaged erythrocytes may lead to iron overload as each unit of erythrocytes contains approximately 250 mg of iron, thus increasing the risk of iron accumulation derived from increased heme degradation. Iron is an essential nutrient for growth and survival, but excess liver iron in particular induces oxidative stress and hepatic fibrogenesis (16). Iron is primarily accumulated in reticuloendothelial cells. The spleen also recycles iron stored in bone marrow and is essential for the removal of stored blood cells, and certain immune functions. Whether and how splenectomy influences iron homeostasis remains unclear.

Given the above-mentioned issues, the current study sought to evaluate how splenectomy impacts iron and CO levels, inflammation, and it decreases portal pressure and slightly decreases hepatic fibroproliferation.

Correspondence to: Professor Zhi-Jun Duan, Department of Gastroenterology, The First Affiliated Hospital of Dalian Medical University, 222 Zhongshan Road, Dalian, Liaoning 116011, P.R. China E-mail: cathydoctor@sina.com

Key words: splenectomy, heme oxygenase-1, iron accumulation, portal vein pressure, bile duct ligation
Materials and methods

Animal care. The Animal Care and Use Committee of Dalian Medical University (Liaoning, China) approved the experimental protocols in accordance with guidelines established by the China Council on Animal Care.

Bile duct ligation and treatment of rats. The fifty healthy male SD rats, weighing 200-220 g, were obtained from the Laboratory Animal Center of Dalian Medical University and were randomly divided into six treatment groups: sham (n=6), BDL (n=10), cobalt protoporphyrin (CoPP) (n=10), zinc protoporphyrin (ZnPP) (n=8), iron-dextran (Fe) (n=8) and splenectomy (n=8). They were housed in a specific pathogen free (SPF) room with a temperature of 24-26°C and relative humidity of 60-65%. Water was given ad libitum.

The rats were fed and housed for three days prior to any experimental protocols. Biliary cirrhosis was induced by BDL (17,18). Five groups underwent BDL together with a sham-operated animal group as a control. The Animal Care and Use Committee of Dalian Medical University approved all surgical procedures. Laparotomy was performed under anesthesia with ether. The bile duct was isolated and double-ligated with a 3-0 silk suture. The abdomen was opened through a midline incision, and the spleen lobe were excised, fixed in 10% neutral formalin solution, and embedded in paraffin. Hematoxylin and eosin (H&E) staining and Van Gieson's (VG) staining were performed according to standard procedures. Lesion severity was graded according to the methods described previously (19). Briefly, tissue sections (4 µm) were treated with HCl (5%) to liberate ferric ions. Samples were then treated with 5% potassium ferrocyanide to produce insoluble ferric ferrocyanide. Slides were counterstained with neutral red. For immunohistochemical examination, deparaffinized sections were incubated with 1% hydrogen peroxide (2 ml) (ultrapure grade; both from Merck KGaA, Darmstadt, Germany) for 20 min at the 120 psi. Resulting products were analyzed directly in the Teflon cartridges.

Histology and immunohistochemistry. Part of the liver and spleen lobe were excised, fixed in 10% neutral formalin solution, and embedded in paraffin. Hematoxylin and eosin (H&E) staining and Van Gieson's (VG) staining were performed according to standard procedures. Lesion severity was graded according to the methods described previously (19). Briefly, tissue sections (4 µm) were treated with HCl (5%) to liberate ferric ions. Samples were then treated with 5% potassium ferrocyanide to produce insoluble ferric ferrocyanide. Slides were counterstained with neutral red. For immunohistochemical examination, deparaffinized sections were incubated with HO-1 antibodies (1:1,000 dilution; Abcam, Cambridge, MA, USA), or anti-α-smooth muscle actin (α-SMA) antibody (1:100 dilution; Boster Biological Technology Ltd., Wuhan, China) and biotinylated secondary antibodies, followed by avidin-biotin-peroxidase complex. Images were analyzed by Image-Pro-Plus 6.0 software (Media Cybernetics, Rockville, MD, USA) to calculate area and mean density of positive expression. Statistical results from five visual fields were averaged from each sample.

Hepatic hydroxyproline content. Liver tissue (100 mg) was prepared for HYP determination according to a modification of the kit method described. The HYP content of the liver served as an indirect measure of tissue collagen content and was expressed as microgram/gram of wet weight (µg/g).

Western blot analysis. The resected liver tissues were extracted with lysis buffer (1% Triton X-100; 50 mmol/l Tris-HCl, pH 7.6; 150 mmol/l NaCl; and 1% protease inhibitor cocktail). Western blot analysis protocols were previously described (20). Western blot analyses were performed with liver homogenates (30 µg protein) using anti-HO-1 antibody (1:2,000 dilution;
Abcam), anti-β-actin antibody (1:500 dilution; Zhongshan Golden Bridge Biotechnology), and secondary anti-rabbit and anti-mouse IgG (1:500 dilution; Biosynthesis Biotechnology, Beijing, China). The intensity of each signal was corrected by the values obtained from the immunodetection of β-actin and the relative protein intensity expressed as fold of the content in the control group.

RNA isolation and gene expression analysis. Total RNA was extracted from livers following a standard guanidinium phenol-chloroform extraction protocol. The quantity of RNA was determined by measuring the optical density at 260 nm (A260 nm = 1 for 40 µg/ml RNA), and RNA purity was assessed by determining the A260/A280 nm ratio (pure RNA: A260/A280 nm = 2.0) using a UV-1206 spectrophotometer (Shimadzu, Kyoto, Japan). An aliquot of each mixture was used for reverse transcription (RT)-PCR amplification using reagents purchased from Takara Bio, Inc. The primer sequences for HO-1 were 5'-ATATCTATACGGCCCTGGAA-3' (forward), 5'-GATGCTCGGGAAGGTGAA-3' (reverse) and the product size was 350 bp, while the primer sequences for β-actin were 5'-GAGGGAAATCGTGCGTGAC-3' (forward), 5'-CTGGAAGGTGGACAGTGAG-3' (reverse) and the product size was 445 bp. PCR products were separated by 2.5% agarose gel electrophoresis. The product bands were photographed and the density of each band was quantified. The results are expressed as the ratio of the band density for the target mRNA to that of β-actin mRNA.

Statistical analysis. All data are presented as the means ± standard deviation. Statistical analysis was performed with SPSS software version 16.0 (IBM, Chicago, IL, USA). Groups were compared using one-way analysis of variance (ANOVA) with Dunnett's multiple comparison test (where applicable). Correlative comparison of two non-hierarchical variances with normal distribution was evaluated by Pearson's test, whereas the Spearman test was used for data with a non-normal distribution. P-values <0.05 were considered to indicate statistically significant differences.

Results

Splenectomy ameliorates liver fibroproliferation and decreases PVP. The common bile duct dilation, ascites, and jaundice were found in all BDL rats at four weeks post-operation, suggesting that BDL was successfully established. Two weeks after the operation, the spleens were much bigger and heavier in the BDL group relative to the sham group (P<0.01), and the body weight (g)/spleen weight (g) ratio was larger in the BDL group (P<0.01) (Table I).

Table I. Body weight and spleen weight in the sham and BDL groups at 2 weeks.

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Spleen weight (g)</th>
<th>Body weight (g)/Spleen weight (g) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>257±6</td>
<td>0.96±0.07</td>
<td>0.37±0.03</td>
</tr>
<tr>
<td>BDL</td>
<td>219±12a</td>
<td>1.49±0.19</td>
<td>0.68±0.04a</td>
</tr>
</tbody>
</table>

αP<0.01 and βP<0.05 vs. sham group. BDL, bile duct ligation.

Table II. Serum biochemical index analysis in different groups (means ± SD, n=6/group).

<table>
<thead>
<tr>
<th></th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>TBIL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>139.00±4.52</td>
<td>36.83±1.72</td>
<td>0.83±0.23</td>
</tr>
<tr>
<td>BDL</td>
<td>255.83±7.55a</td>
<td>49.83±4.36a</td>
<td>11.90±1.96a</td>
</tr>
<tr>
<td>CoPP</td>
<td>406.67±14.81c</td>
<td>72.00±3.52c</td>
<td>13.75±2.23c</td>
</tr>
<tr>
<td>ZnPP</td>
<td>216.00±7.54c</td>
<td>37.17±2.48c</td>
<td>5.53±2.23c</td>
</tr>
<tr>
<td>Fe</td>
<td>309.83±13.61c</td>
<td>43.83±4.96c</td>
<td>13.82±1.94a</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>202.17±6.85cf</td>
<td>37.67±7.06c</td>
<td>5.10±0.48c</td>
</tr>
</tbody>
</table>

αP<0.01 and βP<0.05 vs. sham group; γP<0.01 and δP<0.05 vs. BDL group; εP<0.01 and ΠP<0.05 vs. ZnPP group. BDL, bile duct ligation; AST, aspartate aminotransferase; TBIL, total bilirubin; CoPP, cobalt protoporphyrin; ZnPP, zinc protoporphyrin.
HYP, a product of collagen metabolism, is an amino acid characteristic of collagen. Compared with the BDL group, HYP content was appreciably lower following splenectomy (P<0.01) (Fig. 1Ah). Protein expression levels of α-SMA and TGF-β1 were significantly higher in the BDL group than in the sham group (P<0.01) (Fig. 1B). However, they were much lower in splenectomy-treated rats than in those receiving BDL (P<0.01) (Fig. 1Bg). PVP levels were significantly higher in BDL than in sham-treated rats (P<0.01). PVP levels were reduced following splenectomy, relative to BDL treatment (P<0.01) (Fig. 1Dd). These results suggest that splenectomy not only decreased PVP, but also improved liver function and reduced TGF-β1 secretion, further decreasing hepatic fibrosis.

Figure 1. Assessment of liver fibrosis by different methods. (A) H&E staining and HYP content. (a) Normal lobular architecture in the sham group; (b, c and e) fibrous hyperplasia in the BDL, CoPP and Fe groups; (d and f) less fibrous hyperplasia in the ZnPP and splenectomy groups; (g and h) hepatic fibrosis was assessed using histopathological scores and HYP content (original magnification, x100). (B) Liver sections were stained with α-SMA antibody. (a) There was only slight α-SMA expression around central veins in the sham group. (b, c and e) Elevated expression was observed in the interlobular region in the BDL, CoPP and Fe groups. (d and f) There was less color in the ZnPP and splenectomy groups. The density (mean) of α-SMA was measured by IPP. (g) There was low α-SMA expressed in the splenectomy and ZnPP groups compared with BDL (P<0.01). (h) Serum TGF-β1 was detected by ELISA (original magnification, x100). (C) H&E staining for spleen structure and COHb levels was measured. (a) Macrophages were distributed in the red pulp and marginal zone in normal spleen. (b and c) There were fewer macrophages in the red pulp at two and four weeks. (d) The levels of COHb were quantified (original magnification, x400). (D) VG staining for collagen I in spleen and PVP measurement. (a) Little collagen I deposited in normal spleen. (b and c) More accumulated in the spleen at two and four weeks. (d) PVP levels were measured (original magnification, x400). The data are represented as the means ± SD. *P<0.01, **P<0.05 vs. sham; #P<0.01, ##P<0.05 vs. BDL; $P<0.01, $$$P<0.05 vs. ZnPP. a, Sham; b, BDL; c, CoPP; d, ZnPP; e, Fe; and f, splenectomy.
Splenectomy reduces iron accumulation by inhibiting HO-1 activity. The mRNA and protein expression levels of liver HO-1 increased significantly following BDL treatment compared to sham controls (P<0.01); they were lower in the splenectomy and ZnPP groups (P<0.01) than in the BDL group, but were elevated in the CoPP and Fe groups relative to the BDL group, and decreased in the ZnPP and DFX groups (original magnification, x400). (B) Substantial iron accumulation was present in the Fe group. (g and h) HO-1 expression was observed in the ZnPP and splenectomy groups. (c) HO-1 protein increased in the BDL group relative to sham and were enhanced in the CoPP and Fe groups relative to the BDL group, and decreased in the ZnPP and DFX groups. (C) Serum and liver iron content. Iron content in both liver and serum increased more in the BDL than in the sham group. (a and b) Iron content decreased in the ZnPP and splenectomy groups compared with BDL, and enhanced Fe was observed in the CoPP and Fe groups. The data are represented as the means ± SD. **P<0.01, *P<0.05 vs. sham; ""P<0.01, "P<0.05 vs. BDL; #P<0.01, #P<0.05 vs. ZnPP; a, Sham; b, BDL; c, CoPP; d, ZnPP; e, Fe; and f, splenectomy.

Inhibiting HO-1 expression may improve liver function and decrease fibrosis. On the contrary, it aggravated liver injury (Fig. 1A and B).

Immunostaining of BDL spleen revealed multiple and patchy patterns of HO-1 (brown positive expression). Patterns were visible prominently in red pulp, while those in white pulp were few, if any (Fig. 3A-b). By contrast, the brown stain in the spleen collected from the CoPP and Fe-treated groups, and from macrophages in the red pulp, was markedly elevated. Cross-sections of red pulp appeared to be enlarged compared with analogous cross-sections from the BDL group, which decreased in splenectomy and ZnPP-treated rats (Fig. 3Aa-f).
Changes in HO-1 protein expression levels in the spleen were similar to those in the liver (Fig. 3B).

The iron serum levels in the BDL group were significantly higher than in sham controls (P<0.01), and were markedly lower following splenectomy and ZnPP treatments than after BDL treatment (P<0.01) (Fig. 2Ca). Also, the change of liver iron content correlated with the change of serum iron levels (Fig. 2Cb). We used Prussian blue stain to localize iron accumulation in hepatic and splenic tissue. In the liver and spleen, iron accumulated in Kupffer cells and macrophages in the BDL, CoPP and Fe groups (Figs. 2B and 3D). However, iron staining was essentially absent in the sham, splenectomy, and ZnPP treated groups (Fig. 2Ba, d and f). The areas of iron accumulation in the spleen are shown in Fig. 3C.

These results indicate that the spleen plays an important role in HO-1 expression and iron accumulation, and that splenectomy reduces liver HO-1 expression and reduces iron accumulation. By contrast, enhanced HO-1 expression led to increased accumulation of hepatic iron.

The HO/CO pathway is involved in regulating PVP. Splenic structure was evaluated by H&E staining (Fig. 1C). In normal spleen, macrophages were distributed in red pulp and marginal zones, but were less prevalent in BDL spleen (Fig. 1Ca-c). Collagen deposits were also detected in the spleen. More collagen fibers were found around the splenic corpuscle in the 4th week following BDL treatment than in the 2nd week (Fig. 1Da-c). This may be one factor regulating PVP formation in cirrhotic rats.

The COHb level in arterial blood was significantly higher in the BDL group than in the sham group (P<0.01), significantly lower following splenectomy and ZnPP treatment, and higher in the CoPP and Fe-treated groups than in the BDL group (P<0.01) (Fig. 1Dd). PVP was significantly higher in BDL than in sham...
Splenectomy reduces oxidative stress partly through the HO/CO pathway. MDA levels increased significantly following BDL compared to sham controls, and GSH levels were reduced (P<0.01) (Fig. 3E and F). Splenectomy resulted in a lower level of MDA and elevated GSH levels relative to BDL (P<0.01) (Fig. 3E). This suggests that oxidative stress was activated in cirrhotic rats.

The level of MDA was slightly higher in the CoPP and Fe-treated groups than in the BDL group (P<0.05), but lower in ZnPP-treated rats. GSH levels were significantly enhanced by ZnPP treatment relative to BDL treatment (P<0.01), but decreased following CoPP and Fe treatment (Fig. 3F). Moreover, GSH levels were elevated following splenectomy compared to ZnPP treatment (P<0.01). Markedly, inhibiting HO-1 expression reduced oxidative stress, and induced HO-1 expression and iron accumulation, leading to liver injury.

Correlation analysis revealed that both SOD and MDA were significantly correlated with HYP levels (R=-0.838, 0.871, respectively, P<0.01). These data show that oxidative stress may lead to extracellular matrix (ECM) deposition in the liver and aggravate hepatic fibrosis. These data further indicate that reducing oxidative stress may lighten hepatic fibrosis, partly through the HO/CO pathway after splenectomy.

Discussion

Splenectomy is an effective operation for decreasing PVP. In this study, we found that splenectomy also influences the cirrhotic rat liver by altering HO-1 expression, resulting in iron homeostasis. Two weeks after splenectomy, the levels of AST, ALT and TBIL decreased, as did the serum concentrations of TGF-β1, α-SMA and HYP in the liver. Recent studies suggest that splenectomy may cause anti-inflammatory effects in the portal system in addition to decreasing PVP and reversing hypersplenism, which improves hepatic function (4,5). Moreover, splenectomy may moderate fibrosis by decreasing TGF-β1 secretion. Spleen-derived TGF-β1 plays an inhibitory role in healing hepatic cirrhosis by prohibiting regeneration of damaged liver (21,22). We found it also improved liver function and decreased liver fibrosis following BDL, although splenectomy was initially performed to decrease PVP.

The majority of endogenous CO is catalyzed by inducible expression of HO-1. The release of CO by vascular cells may modulate blood flow and maintain the integrity of the vessel wall (23). It has been suggested that CO interacts with NO, which is a potent activator of soluble guanylate cyclase and a vasodilator (24,25). Indeed, excessive CO production, a consequence of HO-1 overexpression, could play a critical role in modulating vascular tone under different pathological situations (26). COHb levels can be used to estimate HO activity in experimental animals (27). Notably, we observed that upregulated COHb resulted from increased HO-1, which aggravated PVP in BDL rats. Moreover, splenectomy and lower levels of COHb can decrease PVP, with similar results found in the ZnPP treatment group. We reasoned that CO may decrease PVP in early stages, and excessive CO may be harmful by reducing PVP, leading to an unbalanced NO/CO system in the last stage of hepatic fibrosis. It therefore seems appropriate to reduce PVP by decreasing CO.

Our previous study indicated that HO-1 induction can ameliorate immune liver fibrosis (28). Upregulation of HO-1 interferes with chronic inflammation and prevents progression of liver fibrosis in Mdr2 knockout mice (29). Our study found that inhibition of HO-1 could modulate BDL-induced liver fibrosis, which was not in accordance with previous results. We hypothesized that HO-1 plays different roles in the progression of liver fibrosis. In early stage liver fibrosis, inducing HO-1 may have a protective action, but aggravates liver function and PVP during end stage cirrhosis with portal hypertension. Moreover, fibrosis resulting from different animal models may offer another explanation for these results.

We investigated the relationship between HO-1 expression and iron accumulation by inhibiting or inducing HO-1 expression. Inhibiting HO-1 activity reduced iron accumulation in the liver and spleen, and further attenuated hepatic fibrosis. Splenectomy produced similar results, which may be due, partly, to reduced HO-1 expression. Khan et al (30) reported that an increase in HO-1 expression is associated with iron accumulation, and HO-1 activity contributes to increased levels of intracellular labile iron (31). HO-1 gene expression is upregulated by iron, suggesting that degradation of internalized heme may be controlled by a positive feedback loop (32). In the present study, HO-1 was upregulated
following Fe treatment. However, anemia and iron accumulation in the kidney and liver are found in HO-1-deficient mice (33). Absent HO-1 and other HO subtypes, such as HO-2 and HO-3, may play a major role in iron homeostasis. We considered that HO-1 was necessary to form iron homeostasis, and inhibiting it, but not knocking it out, may be useful to reduce iron accumulation.

It has previously been shown that increased deposition of iron in the liver often triggers oxidative stress and inflammation and induces liver cell damage (34). Iron participates in the Fenton and Haber-Weiss reaction, and excessive redox-active iron leads to oxidative stress, with damage to membranes, proteins, and DNA (35). Nontransferrin-bound iron plays a key role in iron overload in severe cirrhosis (36). Our data show that iron accumulation increases oxidative stress and aggravates liver injury in CoPP and Fe-treated groups. This demonstrates that iron may play a pivotal role in hepatic fibrosis.

In our study, iron levels in serum, liver, and spleen all decreased in the splenectomy-treated animals, which showed lower HO-1 than the BDL group. Similar results were found in the ZnPP group. A previous study demonstrated that HO-1 activity is found in the spleen (37). We found enhanced HO-1 expression in the liver and spleen at two and four weeks following BDL. Spleen is a source of HO-1 production and splenectomy could therefore decrease HO-1 generation.

Previous studies have shown that spleen volume negatively correlates with red blood cell and platelet counting (38). Others have noted that splenic hypertrophy partly explains the anemia process, and splenomegaly is widely observed in human fascioliasis (39). These reports indicate that splenomegaly is a key reason for RBC damage and iron production from heme in cirrhotic patients. Splenectomy decreased red blood cell damage and further reduced iron production from heme degradation. This may be a benefit for cirrhotic patients with splenomegaly.

Reactive oxygen species (ROS) may represent a relevant profibrogenic stimulus for hepatic stellate cells (HSCs), promote production of type I collagen, and act as an intracellular signaling mediator of TGF-β (40,41). Splenectomy reduced oxidative stress in BDL rats and further decreased collagen deposition. Of note, however, similar results were found in the ZnPP group due to inhibition of HO-1 expression. HO-1 protected hepatocytes from ethanol-derived oxidative stress via the MAPK/Nrf2 pathway, in primary human hepatocytes (42). Iron accumulation increases liver injury through oxidative stress in nutritional steatohepatitis (43). We thus reasoned that the influences of pro-oxidant activities resulted from iron accumulation and were more effective than the anti-oxidant effects mediated by HO-1.

In conclusion, splenectomy decreases PVP. Moreover, we found it improves liver function partly through the HO/CO pathway during the hepatic cirrhotic process in rats. Inhibition of HO-1 expression by splenectomy led to reduced iron production (Fig. 4). However, we lacked a large-scale clinical trial and in vitro study to better clarify the exact role of the HO/CO pathway in cirrhotic patients following splenectomy, as it is difficult to obtain samples from patients who recover after surgery and who volunteer for a liver biopsy. In addition, we have reason to believe that a reduction in CO due to HO-1 inhibition may be a novel therapeutic option for decreasing PVP. The HO/CO pathway may play a pivotal role in patients with splenectomy to intervene in liver cirrhosis and further reduce PVP.

Acknowledgements

This study was supported by the National Natural Science Foundation of China, no. 30970886, the Technology Project of Dalian, no. 2008E135F193 and the Doctoral Initial Funding of Liaoning Province, no. 20121110.

References


