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Original Article

Metformin reduces intrahepatic fibrosis and intrapulmonary shunts in biliary cirrhotic rats

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Abstract

Background: Liver fibrosis causes portal hypertension which dilates collateral vasculature and enhances extra-hepatic angiogenesis including intrapulmonary shunts, which subsequently complicates with hepatopulmonary syndrome. Metformin is an anti-diabetic agent which possesses anti-inflammation and anti-angiogenesis properties. This study evaluated the effect of metformin treatment on liver and lung in a non-diabetic rat model with biliary cirrhosis induced via common bile duct ligation (CBDL).

Methods: CBDL rats were fed with metformin 150 mg/kg/day during the 8th−28th day post operation. The hemodynamic and biochemistry parameters were tested, and blood gas analysis was performed. The liver and lung were dissected for protein analysis and immuno-histochemical stains. Intrapulmonary shunting degree was determined using color microsphere method.

Results: Metformin treatment neither induced obvious hypoglycemic event nor altered hemodynamics in cirrhotic rats. The plasma levels of alanine aminotransferase were significantly reduced by metformin (control vs. metformin: 269 ± 56 vs. 199 ± 21 IU/L, P = 0.02). Sirius Red stains and CD-68 stains showed that metformin reduced intrahepatic fibrosis and CD-68-positive macrophages. Metformin did not influence hypoxia and intrapulmonary angiogenesis; however, it significantly reduced intrapulmonary shunts (31.7 ± 10.1 vs. 15.0 ± 6.6%, $P = 0.006$.). Furthermore, metformin reduced the protein expressions of COX-2 and PI3K in liver and COX-1 in lung.

Conclusion: Metformin reduced liver injury and improved hepatic fibrosis in cirrhotic rats. It also attenuated the intrapulmonary shunts. However, the effects of metformin on pulmonary angiogenesis and hypoxia were insignificant.

Keywords: Angiogenesis; Hepatopulmonary syndrome; Liver cirrhosis; Metformin

1. Introduction

Metformin is generally accepted as the first-line treatment in type 2 diabetes mellitus patients.[1] It improves peripheral and hepatic sensitivity to insulin, reduces liver gluconeogenesis and enhances the utilization of glucose by peripheral tissues. Apart from hypoglycemic effects, a large body of evidence reveal that metformin has anti-inflammation and anti-angiogenesis properties. The administration of metformin

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prevented and reversed hepatic inflammation in a non-diabetic mouse model of non-alcoholic steatohepatitis.[2] Also, metformin has been noticed in anti-cancer treatment. A nationwide case-control study indicated that metformin reduced the risk of hepatocellular carcinoma in diabetic patients in a dose-dependent manner.[3] Another case-control study showed that diabetes was an independent risk factor for intrahepatic cholangiocarcinoma, and that metformin treatment was associated with a 60% reduction of cancer risk in diabetic patients.[4] The effect of metformin on the prevention and treatment of hepatic cancer comes from, at least in part, inhibition of abnormal angiogenesis.

Hepatopulmonary syndrome (HPS) is a severe complication of liver cirrhosis which is characterized by deoxygenation in cirrhotic patients. Three important components of HPS are: (1) hypoxia with increased alveolar-arterial oxygen gradient ($AaPO_2$); (2) increased intrapulmonary shunts; and (3) chronic liver disease.[5] Recent animal studies show that anti-inflammation and anti-angiogenesis therapies alleviate HPS.[6,7] Using common bile duct ligation (CBDL)-induced biliary cirrhotic rats, researchers have built up a reliable animal model mimicking the clinical presentation of HPS.[8]

The initiation of HPS comes from abnormal vasodilatation, inflammation and angiogenesis in the lung. A recent animal study demonstrated that enhanced chemokine fractalkine expression and signaling contributed to pulmonary monocyte accumulation, angiogenesis and development of HPS.[6] Additionally, Thenappan et al. found that the pulmonary accumulation of CD68-positive macrophages played an important role in development of HPS and speculated depletion of macrophages might be targeted to control HPS.[7] These activated monocytes or macrophages are important modulators to initiate intrapulmonary inflammation and abnormal angiogenesis. The activation of vascular endothelial growth factor (VEGF)-dependent signaling pathways would subsequently increase intrapulmonary shunts then aggravate HPS. We have shown that sorafenib, a tyrosin kinase inhibitor, improves HPS via the attenuation of pulmonary angiogenesis through VEGF/VEGF-R2/Akt pathway inhibition.[9] In addition, we also documented that rosuvastatin alleviated experimental HPS through pulmonary inflammatory angiogenesis blockade, which was related to the down-regulation of tumor necrosis factor-α/nuclear factor kappa B and VEGF/Rho-associated A kinase pathways.[10] Taking the evidence into consideration, the relevant impacts of metformin on intrapulmonary inflammation, angiogenesis, shunting and liver fibrosis is worthy of investigation. In the present study, we evaluated the effect of a 3-week metformin treatment on rats with CBDL-induced experimental HPS.

2. Methods

2.1. Animal model

Male Sprague–Dawley rats weighing 240–270 g at the time of surgery were used for experiments. The rats were housed in plastic cages and allowed free access to food and water. All rats were fasted for 12 h before the operation. Rats with secondary biliary cirrhosis were induced by CBDL. The operation was performed under ketamine anesthesia (100 mg/kg, intramuscularly). A high yield of secondary biliary cirrhosis was noted four weeks after the ligation.[11] To avoid the coagulation defects, CBDL rats received weekly vitamin K injection (50 μg/kg intramuscularly). In all experiments, the principle of laboratory animal care (NIH publication no. 86-23, revised 1985) was followed. This study was approved by the Taipei Veterans General Hospital Animal Committee (approval number: IACUC 2013-161).

2.2. Study protocols

CBDL-induced cirrhotic rats were orally gargled with 150 mg/kg/day metformin or vehicle (0.9% sodium chloride, 1 ml/day, control group) from the 8th to 28th day after CBDL. On the 28th day, the body weight, mortality rate and hemodynamic data were measured (n = 6:6). The blood was collected and protein expressions were examined. Also, liver and lung were examined with hematoxylin and eosin (H&E), Sirius Red, and immunohistochemical stains. Another two parallel groups (n = 7:6) were tested for intrapulmonary shunts using color microsphere method.

2.3. Systemic and portal hemodynamic measurements

The right internal carotid artery of rats was cannulated with a PE-50 catheter that was connected to a Spectramed DTX transducer (Spectramed Inc., Oxnard, CA, USA). Continuous recordings of mean arterial pressure and heart rate were performed on a multi-channel recorder (model RS 3400, Gould, Inc., Cupertino, CA, USA). The abdomen was opened with a mid-line incision, and a mesenteric vein was cannulated with a PE-50 catheter to measure portal pressure.

2.4. Biochemistry and blood gas analysis

The femoral artery and vein of CBDL rats were cannulated with PE-50 catheters one day before experiments. Both catheters were fixed over the back and flushed with a solution contained heparin. The blood was withdrawn from the femoral vein for determining plasma concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, creatinine and glucose levels on the day of experiment. Then, arterial blood was withdrawn from the femoral artery for arterial blood gas analysis. Arterial gas exchange including partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) was evaluated, and the $AaPO_2$ was calculated as $150-(PaCO_2/0.8)-PaO_2$.

2.5. Intrapulmonary shunting analysis

Intrapulmonary shunts were determined using the color-microsphere technique.[9,10] Cross-linked (2.5×10^6) colored microspheres (size range 6.5–10 μm; Interactive Medical Technologies, Los Angeles, CA, USA) were injected through the femoral vein catheter. A reference blood sample was

withdrawn from the femoral arterial catheter beginning at the time of femoral vein injection for a total of 90 s at a constant rate of 1.0 ml/min. Samples of beads before venous injection and reference blood samples were coded. Total numbers of microspheres passing through the pulmonary microcirculation were calculated as reference blood sample microspheres/milliliter × estimated blood volume. The estimated blood volume of each animal was derived from the following formula: blood volume (milliliters) = 0.06 × body weight (g) + 0.77.[12] Intrapulmonary shunting was calculated as an intrapulmonary shunt fraction (%) = (total number of microspheres passing through the pulmonary microcirculation/numbers of total beads injected into the venous circulation) x 100.

2.6. Histopathological and immunohistochemical staining studies of the liver and lung

The liver and lung were dissected free and fixed in 10% formalin solution. The sections were stained with H&E and examined by light microscopy. Liver paraffin section was stained with Sirius Red staining kit (Polysciences, Inc., Warrington, PA, USA) to determine the degree of liver fibrosis. Immunohistochemical staining with anti-CD68 antibody (diluted 1:200, ab31630, Abcam, Cambridge, UK) was performed to determine CD68-positive macrophages in the liver and lung. von Willebrand factor (vWF)-positive cells in the lung tissue were also determined. vWF released by endothelial cells is routinely used to identify angiogenesis in tissue sections, so the numbers of anti-vWF-positive cells indicated the extent of angiogenesis.[13] Immunostaining was performed using the primary antibodies, followed by biotinylated anti-mouse IgG (H + L) (Vector Laboratories, Burlingame, CA, USA) as the second antibody. Detection of biotinylated antibody was performed using the VECTASTAIN®-Elite ABC kit from Vector Laboratories. For chromogen, DAB (3'-diaminobenzidine tetrahydrochloride) was used, which resulted in a brown color at the antigen site. Finally, the sections were counterstained with Mayer's hematoxylin and covered with mounting medium.

2.7. Western blot analysis for protein expressions

The protein extracts of liver and lung were incubated with the primary antibody [anti-endothelial nitric oxide synthase (eNOS), anti-inducible nitric oxide synthase (iNOS) (1:1000; Millipore Corporation, Billerica, MA, USA); anti-cyclooxygenase (COX)-1 (1:1000; Millipore); anti- COX-2 (1:1000; Abcam plc, Cambridge, UK); anti-5'-adenosine monophosphate-activated protein kinase-α 1 (AMPK-α1) (1:500; ab3759; Abcam plc, Cambridge, UK); anti-VEGF (1:1000; Santa Cruz); anti-VEGF receptor 1 (VEGF-R1) (1:1000; Abcam plc, Cambridge, UK); anti-VEGF receptor 2 (VEGF-R2) (1:500; Millipore Corporation, Billerica, MA, USA); anti-phosphoinositide 3-kinases (PI3K) (1:1000; Cell Signaling Technology, Danvers, MA, USA)]. Then the blots were incubated with the secondary antibody (horseradish peroxidase-conjugated goat anti-mouse IgG antibody, Sigma Chemical Co., St. Louis, MO, USA). With a computer assisted video densitometer and digitalizing software (Kodak Digital Science™ ID Image Analysis Software, Eastman Kodak Co., Rochester, NY, USA), the blots were scanned and photographed, then the signal intensity (integral volume) of the appropriate bands was analyzed.

2.8. Drugs

Metformin was purchased from Merck (Merck KGaA, Darmstadt, Germany). All solutions were freshly prepared on the days of experiments.

2.9. Data analysis

The results were expressed as mean ± SD. Statistical analyses were performed using unpaired Student's *t*-test and survival curve analysis using Log-rank test. Results were considered statistically significant at a two-tailed *P* value less of than 0.05.

3. Results

3.1. Mortality rates of metformin- and vehicle-treated CBDL rats

There was no significant difference in mortality rates between metformin- and vehicle-treated (control) CBDL rats [mortality rates were 33.3% (3/9) in the control CBDL group vs. 25% (2/8) in the metformin-treated CBDL group].

3.2. Hemodynamics, biochemistries and blood gas analysis after metformin treatment

Table 1 displays the body weight, hemodynamic change, portal pressure, liver and renal biochemistries and glucose levels of metformin-treated and control CBDL rats. The body weight, mean arterial blood pressure, heart rate and portal

Table 1
Body weight, hemodynamic parameter, biochemistry, arterial gas analysis of CBDL rats receiving vehicle (control) or metformin.

	Control (n = 6)	Metformin (n = 6)
Body weight (g)	377 ± 29	379 ± 43
MAP (mmHg)	119 ± 13	105 ± 9
PP (mmHg)	15.5 ± 2.5	15.9 ± 2.8
HR (beats/min)	339 ± 60	317 ± 33
Glucose (mg/dL)	82 ± 13	93 ± 25
PaO_2 (mmHg)	86.7 ± 5.7	86.3 ± 3.0
$PaCO_2$ (mmHg)	34.4 ± 2.5	37.6 ± 3.5
$AaPO_2$ (mmHg)	20.3 ± 3.8	16.7 ± 3.0
ALT (IU/L)	269 ± 56	199 ± 20*
AST (IU/L)	1125 ± 202	1007 ± 143
TB (mg/dL)	7.9 ± 0.5	7.7 ± 0.8
Cr (mg/dL)	0.4 ± 0.05	0.4 ± 0.06

MAP: mean arterial pressure; PP: portal pressure; HR: heart rate; PaO_2: partial pressure of oxygen; $PaCO_2$: partial pressure of carbon dioxide; $AaPO_2$: alveolar arterial oxygen gradient; AST: aspartate aminotransferase; ALT: alanine aminotransferase; TB: total bilirubin; Cr: creatinine; *$P = 0.026$ between metformin and control group, others $P > 0.05$.

pressure were not significantly different between the metformin-treated and control groups. Metformin did not elicit a significant hypoglycemia in CBDL rats compared to the control group. The plasma levels of creatinine, AST and total bilirubin were also not significantly different. However, the plasma level of ALT was significantly decreased by metformin (control vs. metformin = 269 ± 56 vs. 199 ± 20 IU/L, $P = 0.02$). The PaO_2, $PaCO_2$ and $AaPO_2$ in the arterial blood gas analysis were not significantly different.

3.3. Effects of metformin on the histopathological change of liver

The hepatic H&E staining of CBDL rats showed ballooning change of hepatocytes accompanied by destruction of lobular structure (Fig. 1A). Lymphocyte accumulation in the portal area was compatible with hepatic inflammation. These histological changes were significantly ameliorated by metformin (Fig. 1A and B). The liver tissues of control CBDL rats had prominent CD68-positive staining macrophage infiltration (brown color, Fig. 1C), which were also attenuated by metformin (Fig. 1D). Additionally, Sirius Red staining revealed that metformin significantly attenuated liver fibrosis (red color, Fig. 1E and F).

3.4. Effects of metformin on intrapulmonary inflammation and angiogenesis

There was infiltration by many pulmonary polymorphonuclear cells accompanied by alveolar wall thickening

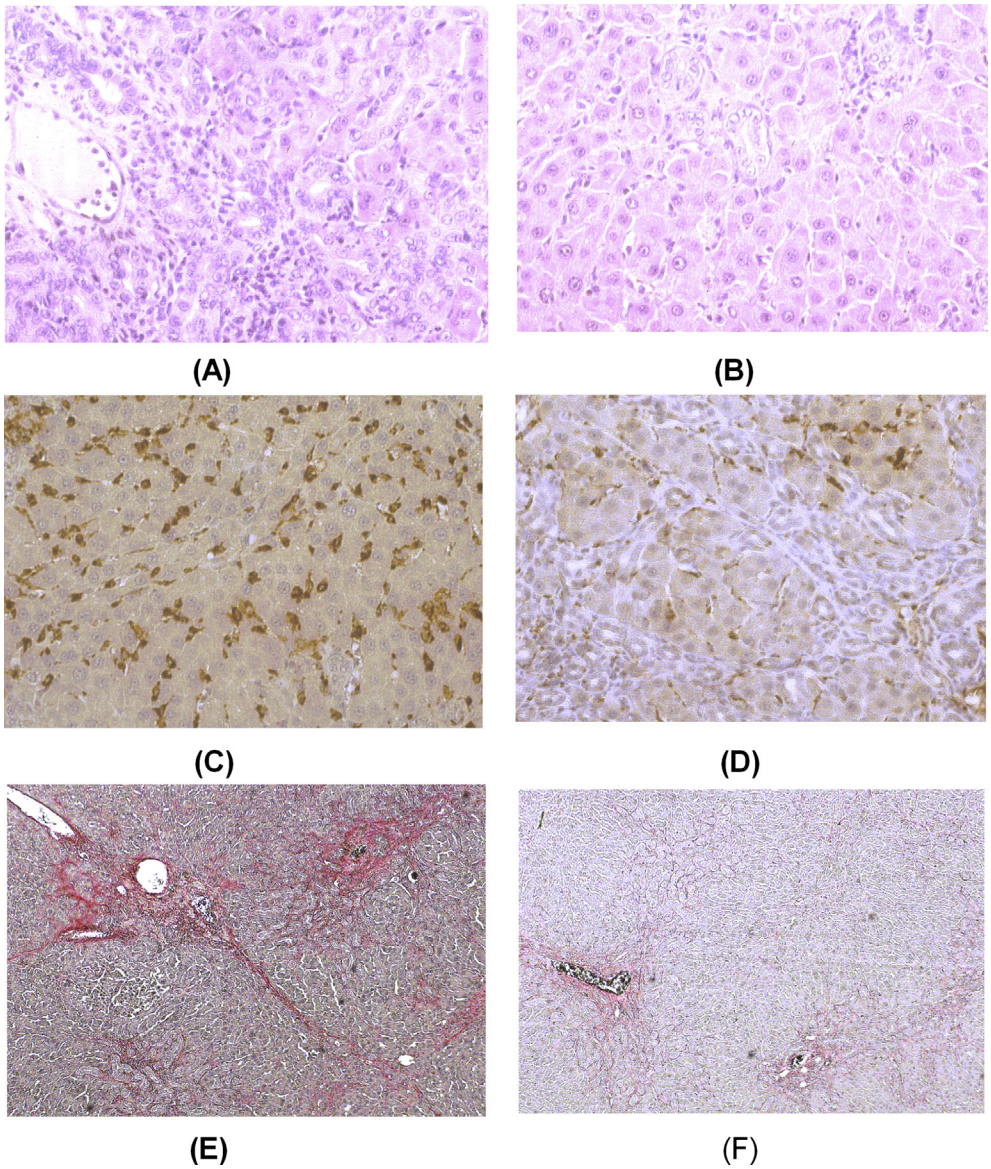

Fig. 1. Metformin alleviated hepatic inflammation and liver fibrosis. The histology of liver showed inflammatory cell infiltration that was ameliorated by metformin (Fig. 1A and B, magnitude 200×). The counts of CD68-positive staining macrophage cells (brown color) were significantly reduced by metformin (Fig. 1C and D, magnitude 200×). Under Sirius Red staining (red color), it was revealed that metformin significantly attenuated liver fibrosis (Fig. 1E and F, magnitude 40×).

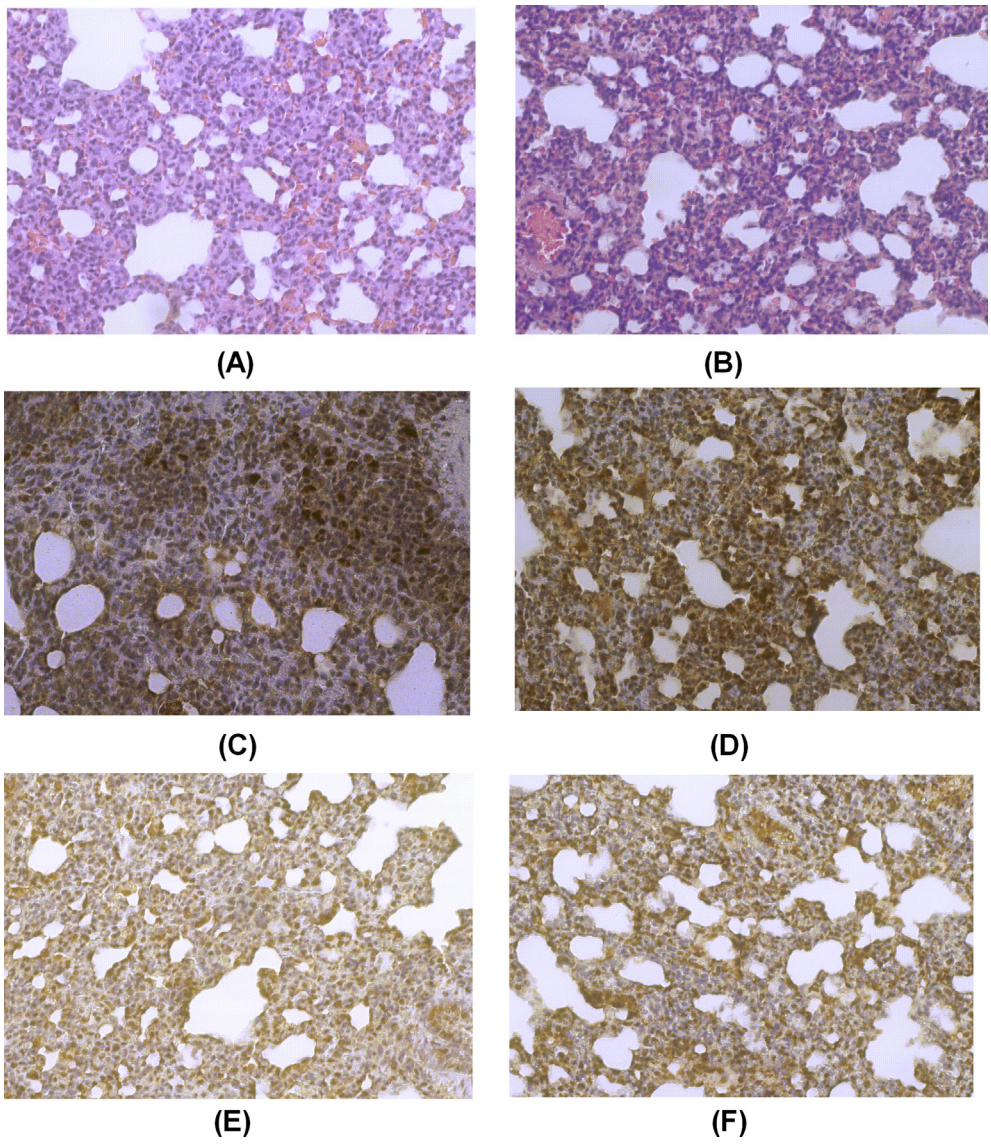

Fig. 2. Metformin neither improved pulmonary inflammation nor diminished intrapulmonary angiogenesis. The intrapulmonary inflammation was similar between metformin-treated and control cirrhotic rats (Fig. 2A and B, magnitude 200×). The lung tissue had prominent CD68-positive staining macrophage infiltration (brown color), which were not attenuated by metformin (Fig. 2C and D, magnitude 200×). The numbers of vWF-staining cells (brown color) were not significantly different between control and metformin-treated CBDL rats (Fig. 2E and F, magnitude 200×).

of both metformin- and vehicle-treated CBDL rats (Fig. 2A and B). The CD68-positive staining macrophage infiltration (brown color, Fig. 2C), was not attenuated by metformin (Fig. 2D). The numbers of vWF-staining cells (brown color) were not significantly different between control and metformin-treated CBDL rats (Fig. 2E and F).

3.5. Protein expressions in the liver of CBDL rats treated by metformin (n = 6) or vehicle (n = 6)

The hepatic protein expressions of PI3K and COX-2 were attenuated by metformin (control vs. metformin: PI3K = 1.56 ± 0.45 vs. 0.89 ± 0.44, P = 0.03; COX-2 = 1.59 ± 0.74 vs. 0.65 ± 0.31, $P = 0.02$; Fig. 3). The VEGF, VEGF-R1, VEGF-R2, COX-1, iNOS, eNOS and AMPK-α1 protein expressions were not influenced by metformin (VEGF = 1.88 ± 0.73 vs. 1.68 ± 0.75; VEGF-R1 = 1.11 ± 0.59 vs. 1.0 ± 0.46; VEGF-R2 = 0.59 ± 0.12 vs. 0.62 ± 0.20; COX-1 = 1.0 ± 0.52 vs. 0.93 ± 0.39; iNOS = 1.09 ± 0.4 vs. 1.52 ± 0.44; eNOS = 0.72 ± 0.41 vs. 0.98 ± 0.93; AMPK-α1 = 1.69 ± 0.47 vs. 1.72 ± 1.23; all $P > 0.05$; Fig. 3).

3.6. Protein expressions in the lung of CBDL rats treated by metformin (n = 6) or vehicle (n = 6)

Metformin significantly down-regulated pulmonary COX-1 protein expression (control vs. metformin: 1.62 ± 0.69 vs. 0.94 ± 0.11; $P = 0.04$; Fig. 4). The VEGF, VEGF-R1, VEGF-

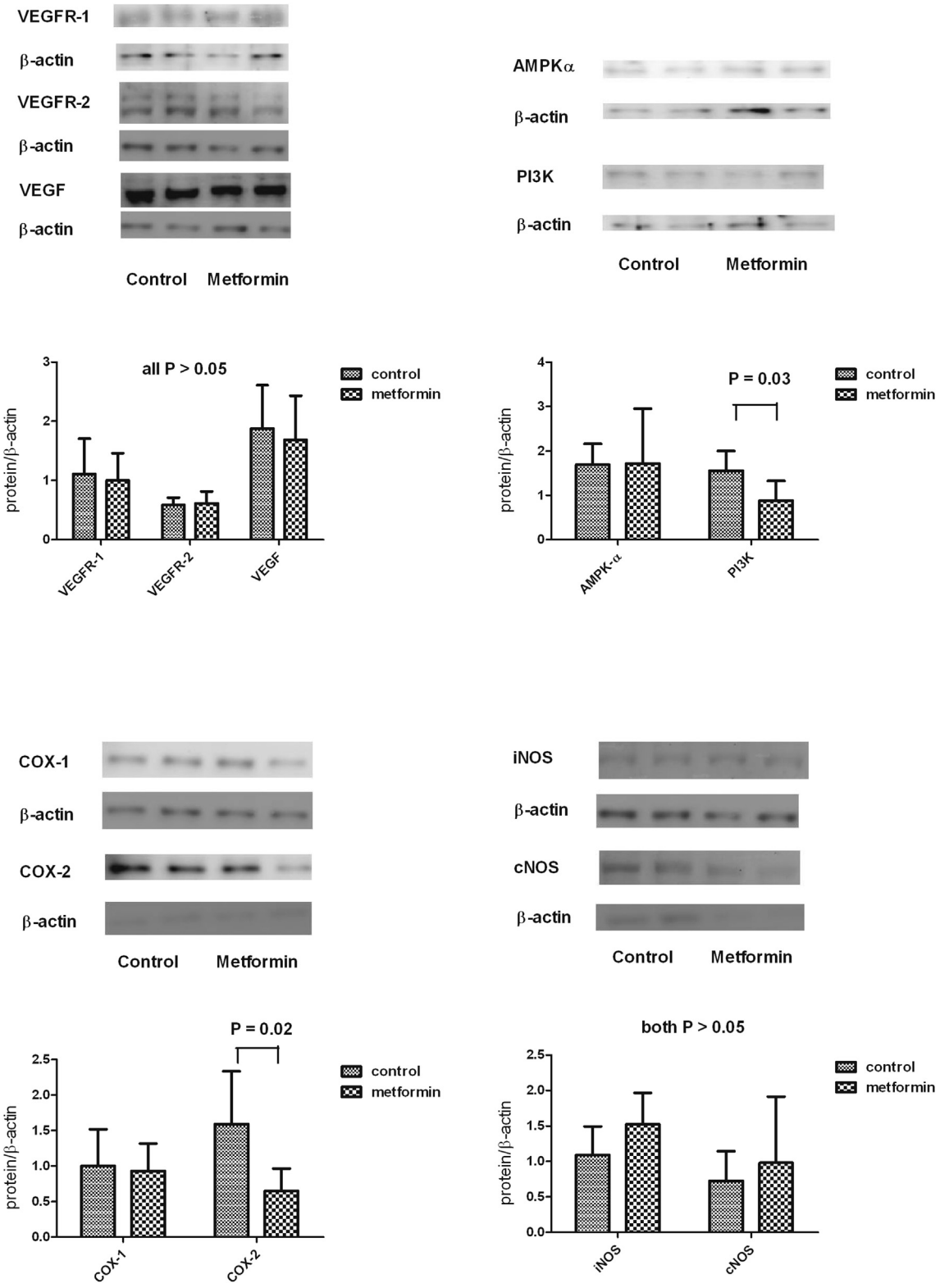

Fig. 3. The hepatic protein expressions of CBDL rats treated by metformin. The densitometric quantification and representative Western blots of PI3K and COX-2 were down-regulated by metformin.

R2, COX-2, iNOS, eNOS, PI3K and AMPK-α1 expressions were not significantly different between metformin-treated and control rats (VEGF = 0.47 ± 0.11 vs. 0.47 ± 0.10; VEGF-R1 = 1.00 ± 0.35 vs. 0.94 ± 0.54; VEGF-R2 = 0.53 ± 0.10 vs. 0.66 ± 0.2; COX-2 = 0.96 ± 0.41 vs. 0.80 ± 0.25; iNOS = 0.77 ± 0.34 vs. 1.05 ± 0.23; eNOS = 0.81 ± 0.28 vs. 0.86 ± 0.28; PI3K = 1.03 ± 0.33 vs. 1.06 ± 0.24; AMPK-α1 = 0.97 ± 0.30 vs. 0.94 ± 0.26; all $P > 0.05$; Fig. 4).

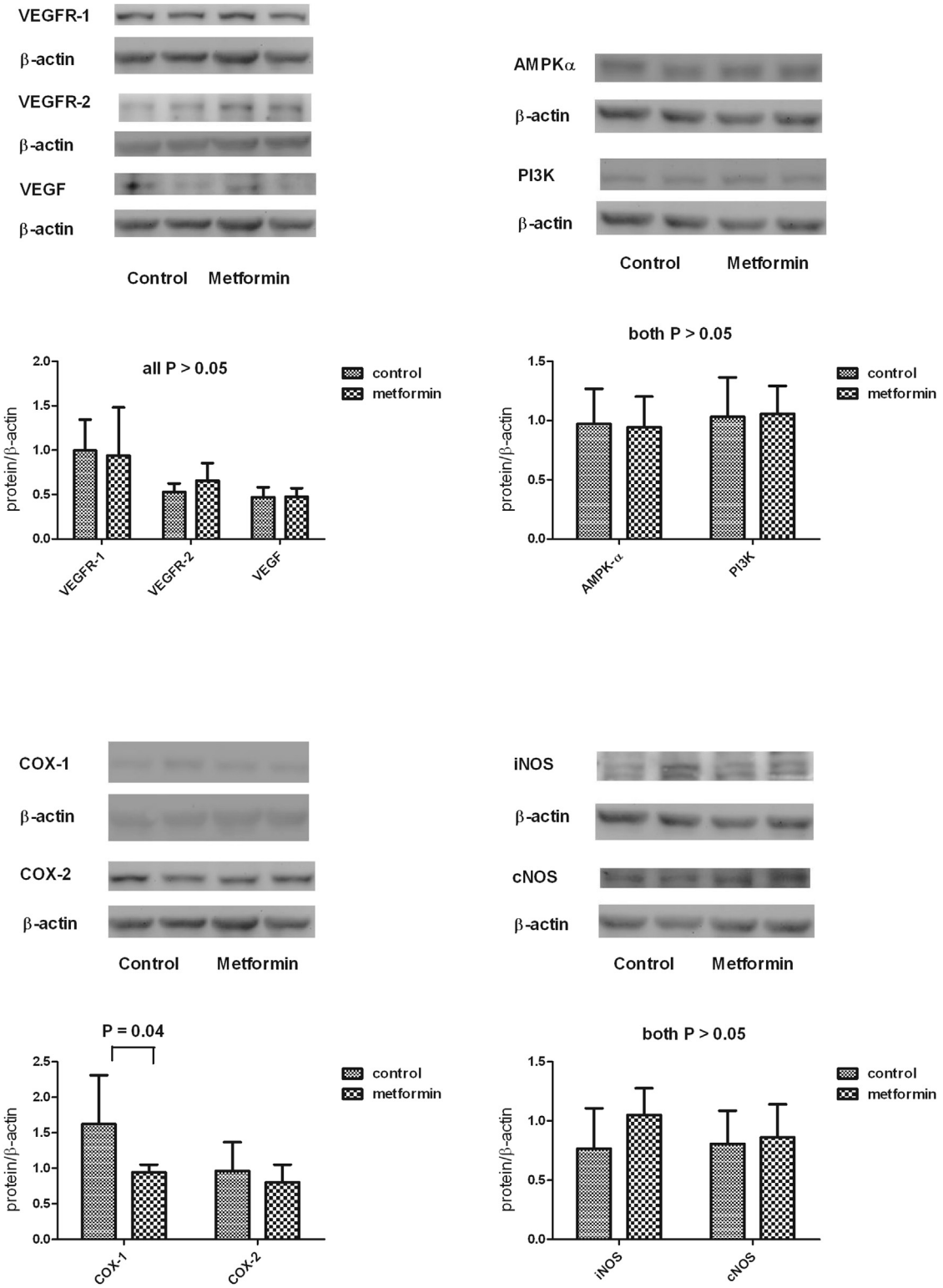

Fig. 4. The pulmonary protein expressions of CBDL rats treated by metformin. The pulmonary COX-1 protein expression was attenuated by metformin.

3.7. Intrapulmonary shunts of metformin-treated and control CBDL rats (control vs. metformin; n = 7:6)

The intrapulmonary shunts were significantly decreased by metformin as compared with the control group (control vs. metformin: 31.7 ± 10.1 vs. 15.0 ± 6.6%; $P = 0.006$; Fig. 5).

4. Discussion

In accordance with our previous studies,[9,10] CBDL rats developed significant liver fibrosis with hypoxia, which mimics the clinical presentation of HPS in cirrhotic patients. Although metformin significantly reduced intrapulmonary

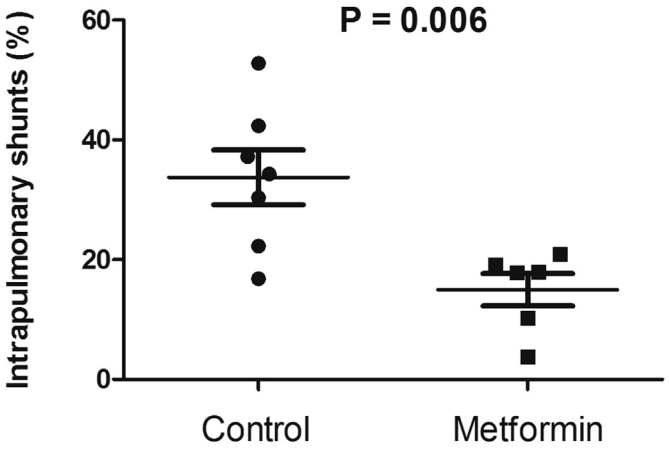

Fig. 5. Metformin reduced intrapulmonary shunts of cirrhotic rats. The intrapulmonary shunts were significantly decreased by metformin as compared with the control group with CBDL-induced HPS.

shunts in the present study, it neither improved hypoxia nor reduced $AaPO_2$, indicating a multifactorial nature of HPS that could not be reversed merely by alleviating shunts. On the other hand, our data support previous findings focusing on the beneficial effect of metformin in improving hepatic fibrosis and inflammation.[14]

We found that a 21-day metformin (150 mg/kg/day) treatment did not elicit hemodynamic changes in cirrhotic rats. In addition, metformin did not cause detrimental effects such as increased mortality, hypoglycemia or renal function deterioration. Taking the poor glycogen storage in the cirrhotic liver into consideration, hypoglycemia can be a concern for cirrhotic patients taking metformin. However, clinical observations have indicated that metformin rarely induced hypoglycemia in cirrhotic patients.[15] Our results also suggest that metformin therapy could be relatively safe in cirrhosis.

The 21-day 150 mg/kg/day metformin treatment in the present study did not reduce portal pressure. A recent study showed that a 7-day 300 mg/kg/day metformin treatment reduced portal pressure and hepatic vascular resistance in cirrhotic rats.[14] The discrepant results might be related to different doses. We chose 150 mg/kg/day in the present study based upon a previous report showing that a dose below 200 mg/kg/day metformin did not elicit an increase of mortality or morbidity of rats.[16]

Our data showed that metformin significantly reduced the plasma level of ALT in cirrhotic rats. Metformin has been demonstrated to improve hepatic function in patients with hepatitis C-related liver cirrhosis.[17] The anti-inflammatory action of metformin results from AMPK activation.[18] Metformin activates AMPK to inhibit interleukin-6-stimulated inflammatory response in human liver cells.[19] In addition, in a galactosamin-induced hepatic injury animal model, metformin exerted AMPK-dependent protective effects.[20] However, our data showed metformin administration did not upregulate AMPK, although the hepatic inflammation was alleviated, as evidenced by improved liver histology and diminished CD-68 positive macrophages. Furthermore, we found that metformin attenuated hepatic PI3K and COX-2 protein expression. Consistently, previous studies have indicated AMPK-independent pathways; the hepatoprotective effect of metformin against alcohol injury comes from plasminogen activator inhibitor (PAI)-1 inhibition, independent of AMPK activation.[21] PAI-1 is an acute-phase protein known to correlate with hepatic fibrosis. Using a CBDL mice model, Bergheim et al. reported that PAI-1 played a central role in mediating fibrosis during cholestasis.[22] In addition, in agreement with our findings, Woudenberg-Vrenken et al. found that metformin dose-dependently alleviated bile acid-induced hepatocyte damage through the PI3K/Akt pathway, which does not require AMPK-signaling.[23] Taken together, our data demonstrate an AMPK-independent pathway against hepatic inflammation exerted by metformin.

Regarding liver fibrosis, Tripathi et al. showed that a 7-day 300 mg/kg/day metformin treatment reversed hepatic fibrosis in CCl_4-induced but not in CBDL-induced cirrhotic rats.[14] The researchers proposed that the CBDL model is characterized by a very rapid progression of fibrosis with no possibility of spontaneous regression due to the persistence of bile duct obstruction, therefore metformin only elicited negligible effect. However, our data showed a 21-day 150 mg/kg/day metformin treatment improved liver fibrosis, as evidenced by Sirius Red staining. The discrepant data might be ascribed to different treatment regimens. Indeed, another study indicated that metformin inhibits hepatic stellate cells proliferation via suppression of reactive oxygen species production, followed by liver fibrosis alleviation.[24]

Metformin reduces endotoxin-induced acute lung injury, both *in vivo* and *in vitro*.[25] Metformin use is also associated with improved dyspnea in patients with chronic obstructive pulmonary disease.[26] In rats with bleomycin-induced acute lung injury and cultured fibroblasts, metformin ameliorates both airway inflammation and remodeling.[27] Therefore, we postulated initially that metformin might improve hypoxia and pulmonary inflammation in rats with CBDL-induced HPS. However, the results did not fully support our hypothesis. The possible causes could be: (1) ineffective dose, (2) short treatment duration, and (3) late treatment. Whether a higher dose (such as 300 mg/kg/day), a longer duration and/or early administrations of metformin (since the first day of or before CBDL) could be effective deserves further evaluation.

Metformin has a dual effect of pro-angiogenesis and anti-angiogenesis. Metformin significantly decreased angiogenesis in granulomatous tissue of rats and inhibited VEGF expression and endothelial cell migration through the AMPK pathway.[28] In contrast, in pulmonary artery endothelial cells isolated from fetal lambs with pulmonary hypertension, metformin upregulated AMPK and eNOS expressions, which further enhanced pulmonary angiogenesis.[29] In the present study, we found that metformin treatment had a neutral effect of pulmonary angiogenesis in cirrhotic rats. The protein expressions of VEGF, VEGF-R1 and VEGF-R2 were not influenced by metformin. In addition, the counts of vWF-positive endothelial cells were similar between metformin-treated and control rats. However, metformin significantly decreased intrapulmonary shunts in CBDL rats. The attenuation of intrapulmonary shunts might be related to direct pulmonary

vasoconstriction or inhibition of angiogenesis. Since metformin did not significantly influence pulmonary angiogenesis, the diminished intrapulmonary shunts might come from the intrapulmonary vasoconstriction. Metformin significantly attenuated COX-1 protein expression in the present study. It has been reported that COX-1 inhibition decreased the amount of vasodilatory prostaglandin, such as prostacyclin. Prostacyclin is a potent vasodilator affecting vascular tone in cirrhosis.[30] It is thus inferred that COX-1 inhibition by metformin may participate in alleviating the degree of intrapulmonary shunting via intrapulmonary vasoconstriction.

In conclusion, metformin does not cause detrimental effects in CBDL-induced cirrhotic rats, including hypoglycemia and renal function deterioration. Metformin alleviates hepatic damage in cirrhotic rats. Furthermore, although metformin does not improve hypoxia, it reduces intrapulmonary shunts in HPS rats. Thorough description of the mechanism awaits further investigation.

Acknowledgments

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