Effects of dipeptidyl peptidase-4 inhibition on portal hypertensive and cirrhotic rats

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Abstract

Background: Portal hypertension is a pathophysiological abnormality with distinct vascular derangements associated with liver cirrhosis. Dipeptidyl peptidase-4 (DPP-4) inhibitors are antidiabetic agents which exert pleiotropic vascular effects, but their relevant impact on portal hypertension and liver cirrhosis remains unclear. This study aims to clarify this issue.

Methods: Rats receiving partial portal vein ligation (PVL) and common bile duct ligation (BDL) served as experimental models for portal hypertension and cirrhosis, respectively. After linagliptin (a DPP-4 inhibitor) treatment, the survival rate, hemodynamics, biochemistry parameters and liver histopathology were evaluated. In addition, the collateral vascular responsiveness and severity of portal-systemic shunting were examined. mRNA and protein expression in the vasculature and liver were also examined.

Results: Linagliptin significantly reduced portal pressure (control vs linagliptin: 12.9 ± 1.2 vs 9.1 ± 2.0 mmHg, $p = 0.001$) and upregulated nitric oxide synthase expression in the collateral vessel, superior mesentery artery, and liver of PVL rats. However, the portal hypotensive effect was insignificant in BDL rats. Glucose plasma levels, liver and renal biochemistry parameters were not significantly altered by linagliptin. The degree of portal-systemic shunting and collateral vascular responsiveness were also not significantly altered by linagliptin treatment. Linagliptin did not improve liver fibrosis and hepatic inflammation in BDL rats.

Conclusion: DPP-4 inhibition by linagliptin reduced portal pressure in portal hypertensive rats but not in cirrhotic rats. It may act by decreasing intrahepatic resistance via upregulation of hepatic nitric oxide synthase in portal hypertensive rats.

Keywords: Linagliptin; Liver cirrhosis; Portal hypertension

1. INTRODUCTION

In chronic liver disease, an increase in intrahepatic resistance develops due to liver inflammation and fibrosis, which impedes hepatic blood flow and subsequently leads to portal hypertension and the development of portosystemic collateral vascular bed.[1] An increased release of extrahepatic nitric oxide (NO, a potent vasodilator) aggravates the portosystemic collaterals.[2] At the same time, a decreased amount or bioavailability of intrahepatic NO causes vasoconstriction and exacerbates portal hypertension.[3] The imbalance in intrahepatic and extrahepatic NO production and bioavailability is therefore attributed for the complicated pathophysiology of liver cirrhosis.[4] Blockade of NO synthase significantly ameliorates portal-systemic shunting.[5] However, systemic NO blockade raises concerns regarding a further depletion in intrahepatic NO and an exacerbation of portal hypertension. The regional difference in NO availability is the most challenging issue that negatively impacts the treatment efficacy of pharmacological agents.

Dipeptidyl peptidase-4 (DPP-4) inhibitors enhance the effect of glucagon-like peptide-1 (GLP-1), which leads to the secretion of insulin and the reduction of glucose plasma levels. Nowadays, DPP-4 inhibitors are widely used for the treatment of diabetes mellitus.[6] In addition to lowering blood glucose levels, DPP-4 inhibitors also exert pleiotropic vascular effects. DPP-4 has widespread organ distribution throughout the body and DPP-4 blockade promotes endothelial cell function via endothelial NO synthase (eNOS) signaling, which is mediated by both GLP-1–dependent and GLP-1–independent mechanisms.[7] DPP-4 inhibition also reduces blood pressure and vascular inflammation in hypertensive rats by increasing NO bioavailability.[8] Besides, acute administration of DPP-4 inhibitors dilates aortic segments through increased NO release.[9]

Upregulated DPP-4 expression in cirrhotic liver and elevated serum levels of DPP-4 in cirrhotic patients have been reported.[10,11] In addition, emerging studies indicate that DPP-4 is involved in the development of various chronic liver diseases, such as hepatitis C virus infection, nonalcoholic fatty liver disease, and hepatocellular carcinoma.[12] A retrospective review of 459 patients with

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type 2 diabetes who were prescribed DPP-4 inhibitors showed that DPP-4 inhibitors ameliorated liver dysfunction.[13] In addition, DPP-4 inhibitors ameliorated liver fibrosis via suppression of hepatic stellate cell proliferation and collagen synthesis.[14]

The effects of DPP-4 inhibition on portal hypertension, portosystemic collaterals and cirrhotic liver are unknown, so this study was designed to test the relevant effects of DPP-4 inhibition on rats with partial portal vein ligation (PVL)–induced portal hypertension and common bile duct ligation (BDL)–induced biliary cirrhosis.

2. METHODS

2.1. Animal model for portal hypertension

Male Sprague-Dawley rats (280–300 g) were caged at 24°C with a 12-hour light-dark cycle and free access to food and water until the time of the experiments. Survival surgery and hemodynamic studies were performed under Zoletil (tiletamine + zolezepam) anesthesia (50 mg/kg, intramuscularly). Portal hypertension was induced by PVL as previously described.[15] Sham operations without ligation of the portal vein were conducted as the surgical control.

2.2. Animal model for biliary cirrhosis

Male Sprague-Dawley rats weighing 280 to 300 g at the time of surgery were used for the experiments. Rats with secondary biliary cirrhosis were induced by BDL.[16] A high yield of secondary biliary cirrhosis was noted 4 weeks after ligation.[17] To avoid coagulation defects, BDL rats received weekly vitamin K injections (50 μg/kg intramuscularly). The control group received a sham operation without ligation of the common bile duct.

2.3. Experimental design

In the first series, a dose-finding study was performed to test the portal hypotensive effects of linagliptin, a DPP-4 inhibitor, on PVL rats. One day before PVL operation, the rats were randomly allocated to receive oral gavage of normal saline (vehicle control), 3 mg/kg, 10 mg/kg, or 30 mg/kg linagliptin for 10 consecutive days. On the tenth day, body weight (BW), mean arterial pressure (MAP), heart rate (HR), portal venous pressure (PP), and fasting blood glucose levels were measured. The optimal dose of linagliptin treatment was then used for the following experiments.

In the second series, the effect of linagliptin on PVL-induced portal hypertensive rats was examined. One day before PVL or the sham operation, the rats were randomly allocated to receive either oral gavage of linagliptin or normal saline for 10 consecutive days. On the tenth day, BW and hemodynamic data including MAP, HR, PP, superior mesenteric arterial blood flow (SMAf), portal venous blood flow (PVf), cardiac index (CI), and systemic vascular resistance (SVR) were collected. Blood was drawn to measure the plasma levels of glucose, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin at the end of the experiments. The mRNA expression of eNOS and GLP-1 were determined in the SMA and splenorenal shunt (the most prominent collateral vessel of portal hypertensive rats) and the hepatic protein expression of eNOS and GLP-1 were also determined.

In the third series, the effect of 10-day linagliptin treatment on portal-systemic shunting of portal hypertensive rats was evaluated. Portosystemic shunting was measured using the color microsphere method.

In the fourth series, the vascular responsiveness of collateral vessels as influenced by linagliptin preincubation was assessed in portal hypertensive rats. Using an in situ perfusion model, the vascular responsiveness of collateral vascular bed to acetylcholine was evaluated in rats on the tenth day post-PVL.

In the fifth series, the effect of linagliptin on BDL-induced cirrhotic rats was evaluated. Two weeks post-BDL or sham operation, rats were randomly allocated to receive either oral gavage of linagliptin or normal saline for 2 consecutive weeks. On the 28th day, measurements of BW, portal and systemic hemodynamics and biochemistry parameters were taken. The protein expression of NO synthase and GLP-1 and the histopathological changes of the liver were examined.

The principles of laboratory animal care (NIH publication no. 86-23, revised 1985) were followed. This study was approved by the Taipei Veterans General Hospital Animal Committee (IACUC 2016-059).

2.4. Measurement of systemic and portal hemodynamics

The right femoral artery and mesenteric vein were cannulated with PE-50 catheters connected to a Spectramed DTX transducer (Spectramed Inc., Oxnard, CA, USA). Continuous recordings of MAP, HR, and PP were taken on a multichannel recorder (model RS 3400, Gould Inc., Cupertino, CA, USA). Cardiac output was measured by thermodilution, as previously described.[18] CI (mL/min/100 g BW) was calculated as cardiac output per 100 g BW of the rat. SVR (mmHg/mL/min/100 g BW) was calculated by dividing the MAP by the CI.

2.5. Portal venous flow and superior mesenteric artery flow measurements

Measurements of PVf (mL/min/100 g BW) and SMAf (mL/min/100 g BW) were taken using a nonconstrictive perivascular ultrasonic transit-time flow probe (lRB, 1-mm diameter; Transonic Systems, Ithaca, NY, USA).[19]

2.6. Portosystemic shunting analysis

Portosystemic shunting was determined using color microspheres.[20] The degree of portosystemic shunting was calculated as the number of microspheres in the lung divided by the sum of microspheres in the liver and lung.

2.7. In situ perfusion study of portosystemic collateral vascular bed

In situ perfusion was performed as described in our previous report.[15] The collateral vessels were preincubated by vehicle (Krebs solution) or linagliptin (10^{-6} M) for 25 minutes, then preconstricted by 10^{-6} M norepinephrine. After the perfusion pressure was stabilized, escalated concentrations of acetylcholine (10^{-9} to 10^{-6} M) were added in the perfusate to evaluate the vasodilatory responsiveness of the collateral vessels. Only one concentration-response curve was performed for each preparation. After testing with the experimental agents, the contracting capability of the collateral vessels was checked with a 125-mmol/L potassium chloride solution at the end of the experiments.

2.8. Total RNA isolation and real-time quantitative polymerase chain reaction

Total RNA was extracted from the SMA and splenorenal shunt using the RNeasy Mini kit (Qiagen GmbH, Hilden, Germany). Real-time quantitative polymerase chain reaction was then performed on a LightCycler (LightCycler 480, Roche Diagnostics, Mannheim, Germany) and a standard LightCycler amplification cycle protocol was established for each gene. The internal housekeeping gene β-actin was used as the control. The primer sequences were as follows: β-actin forward, 5′-CGCCCTAGGCACCAGGGTG-3′ and reverse, 5′-GCTGGGGTGTTGAAGGTCTCAAA-3′; eNOS forward, 5′-GGAAGTAGCCAATGCAGTGAA-3′ and reverse, 5′-GCCAGTCTCAGAGCCATACA-3′; GLP-1 forward, 5′-CAGAAGTTGGTCGTGAGGGA-3′ and reverse, 5′-GCCTTTCACCAGCCAAGCAA-3′.

Table 1

BW, hemodynamic parameters, and fasting blood glucose level of PVL rats with different doses of linagliptin administration

	Control (vehicle) (n = 7)	Linagliptin (3 mg/kg) (n = 6)	Linagliptin (10 mg/kg) (n = 6)	Linagliptin (30 mg/kg) (n = 7)
BW (g)	311 ± 14	294 ± 39	294 ± 13	295 ± 28
MAP (mmHg)	127 ± 22	130 ± 22	116 ± 17	130 ± 11
PP (mmHg)	12.9 ± 1.2	10.4 ± 1.6	8.6 ± 2.4[a]	9.1 ± 2.0[a]
HR (beats/min)	386 ± 21	400 ± 30	414 ± 20	408 ± 29
Glu (mg/dL)	119 ± 15	104 ± 5	117 ± 10	111 ± 9

BW = body weight; Glu = glucose; HR = heart rate; MAP = mean arterial pressure; PP = portal pressure; PVL = partial portal vein ligation.
[a]$p < 0.05$ compared with the control group.

2.9. Western blot analysis

Proteins were extracted from the liver and incubated with primary antibodies against antiphosphorylated eNOS (1:500; Transduction Laboratories, Lexington, UK) and anti–GLP-1 (1:1000; Abcam plc, Cambridge, UK). They were then incubated with secondary antibodies (horseradish peroxidase–conjugated goat anti-mouse IgG antibody; Sigma Chemical Co., St. Louis, MO, USA). Subsequent detection of the specific proteins was performed by enhanced chemiluminescence (BCIP/NBT solution, Amresco Co., Solon, OH, USA). Computer-assisted video densitometer and digitalized software (Kodak Digital Science™ ID Image Analysis Software, Eastman Kodak Co., Rochester, NY, USA) was used to scan and photograph the blots, and the signal intensity (integral volume) of the appropriate bands was analyzed.

2.10. Drugs

Linagliptin was kindly provided by Boehringer Ingelheim International GmbH USA. The reagents for preparing Krebs solution, norepinephrine and acetylcholine, were purchased from Merck KGaA (Munich, Germany). All solutions were freshly prepared on the day of each experiment.

2.11. Statistical analysis

Results are expressed as the mean ± standard deviation. Statistical analyses were performed using Student's *t* test for hemodynamics, biochemistry, shunting degree, mRNA and protein expression. Survival curve analysis was performed using a log-rank test. Two-way analysis of variance with Bonferroni's correction of multiple means was used for the comparison of vascular responsiveness. Results are considered statistically significant with a p value <0.05.

3. RESULTS

3.1. Survival rates and complications

All sham-operated and PVL rats survived. The BDL group had a significantly higher mortality rate compared with the sham-operated group (BDL vs control: 28.6% [2/7] vs 0% (0/7), $p < 0.05$). There was no significant difference in the mortality rates between the BDL and BDL + linagliptin groups (BDL vs BDL + linagliptin: 28.6% [2/7] vs 14.3% [1/7], $p > 0.05$). No significant adverse effects were observed throughout the experimental period.

3.2. Portal hypotensive effects of different dosages of linagliptin treatment in PVL rats

Table 1 shows the hemodynamic parameters and fasting blood glucose of PVL rats treated with different dosages of linagliptin. The 10-day 10 and 30 mg/kg linagliptin treatment significantly decreased the PP of PVL rats without significantly altering the BW, MAP, HR, and plasma levels of fasting glucose (control as vehicle vs 3 mg/kg linagliptin vs 10 mg/kg linagliptin vs 30 mg/kg linagliptin: 12.9 ± 1.2 vs 10.4 ± 1.6 vs 8.6 ± 2.4 vs 9.1 ± 2.0 mmHg; control vs 10 mg/kg linagliptin and control vs 30 mg/kg linagliptin, $p < 0.05$). Considering the different degrees of liver injury and liver reserve of the PVL and BDL animal models, we chose 30 mg/kg linagliptin as the dosage for the following PVL experiments and 10 mg/kg linagliptin as the dosage for the BDL experiments.

3.3. Impact of linagliptin treatment on rats with PVL-induced portal hypertension

Table 2 shows the hemodynamic and biochemistry parameters in the control (sham + vehicle), PVL (PVL + vehicle), and PVL +

Table 2

BW, hemodynamic parameters, and biochemistry data of PVL rats with or without linagliptin (30 mg/kg) treatment

	Sham + vehicle (n = 8)	PVL + vehicle (n = 7)	PVL + linagliptin (n = 7)
BW (g)	302 ± 15	311 ± 14	295 ± 28
MAP (mmHg)	121 ± 10	127 ± 22	130 ± 11
PP (mmHg)	8.8 ± 1.1	12.9 ± 1.2[a]	9.1 ± 2.0[b]
HR (beats/min)	376 ± 27	386 ± 21	408 ± 29
PVf (mL/min/100 g)	6.4 ± 0.6	11.7 ± 1.3[a]	12.5 ± 2.5[a]
SMAf (mL/min/100 g)	4.8 ± 0.7	7.2 ± 1.8[a]	7.8 ± 1.5[a]
SVR (mmHg/mL/min/100 g)	2.9 ± 0.4	4.7 ± 1.7[a]	4.8 ± 1.0[a]
CI (mL/min/100 g)	42.3 ± 5.5	29.4 ± 6.2[a]	27.8 ± 4.1[a]
ALT (IU/L)	60 ± 20	67 ± 11	60 ± 22
AST (IU/L)	114 ± 46	157 ± 29	150 ± 31
TB (mg/dL)	0.04 ± 0.02	0.05 ± 0.02	0.07 ± 0.02
Cr (mg/dL)	0.47 ± 0.07	0.53 ± 0.05	0.47 ± 0.06
Glu (mg/dL)	107 ± 14	119 ± 15	111 ± 9

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BW = body weight; CI = cardiac index; Cr = creatinine; Glu = glucose; HR = heart rate; MAP = mean arterial pressure; PP = portal pressure; PVL = partial portal vein ligation; PVf = portal venous blood flow; SMAf = superior mesenteric arterial blood flow; SVR = systemic vascular resistance; TB = total bilirubin.
[a]$p < 0.05$ compared with the sham + vehicle group.
[b]$p < 0.05$ compared with the PVL + vehicle group.

linagliptin groups. Rats in the PVL and PVL + linagliptin groups had significantly elevated PVf, SMAf, and SVR compared with the control rats (PVf, control vs PVL vs PVL + linagliptin: 6.4 ± 0.6 vs 11.7 ± 1.3 vs 12.5 ± 2.5 mL/min/100 g; control vs PVL, PVL + linagliptin, $p < 0.05$; SMAf, 4.8 ± 0.7 vs 7.2 ± 1.8 vs 7.8 ± 1.5 mL/min/100 g; control vs PVL, PVL + linagliptin, $p < 0.05$; SVR, 2.9 ± 0.4 vs 4.7 ± 1.7 vs 4.8 ± 1.0 mmHg/mL/min/100 g; control vs PVL, PVL + linagliptin, $p < 0.05$). However, linagliptin treatment did not significantly elevate PVf, SMAf, and SVR in the PVL rats ($p > 0.05$). The CI was decreased in PVL rats with and without linagliptin treatment compared with the control group (control vs PVL vs PVL + linagliptin: 42.3 ± 5.5 vs 29.4 ± 6.2 vs 27.8 ± 4.1 mL/min/100 g; control vs PVL, PVL + linagliptin, $p < 0.05$). It is worth noting that the PP was elevated in PVL rats compared with the control group but this was reversed after linagliptin treatment (control vs PVL vs PVL + linagliptin: 8.8 ± 1.1 vs 12.9 ± 1.2 vs 9.1 ± 2.0 mmHg; control vs PVL, $p < 0.05$; PVL vs PVL + linagliptin, $p < 0.05$). There was no significant difference in BW, MAP, HR, ALT, AST, total bilirubin, creatinine, and glucose levels among the groups.

3.4. Portal-systemic shunting in PVL rats with or without linagliptin treatment (n = 6:6)

Linagliptin did not significantly influence the degree of portosystemic collateral shunting in PVL rats (control as vehicle vs linagliptin: 30.4 ± 10.9 vs 31.5 ± 11.2%, $p > 0.05$; Fig. 1).

3.5. Effect of linagliptin on portosystemic collateral vascular responsiveness in PVL rats (n = 5:5)

Fig. 2 depicts the percentage of collateral vessel relaxation to acetylcholine at concentrations of 10^{-9} to 10^{-6} M (based on 100% relaxation to 10^{-6} M acetylcholine) after Krebs solution (control) or linagliptin preincubation in PVL rats. The relaxation of collateral vessels was not altered by linagliptin preincubation in PVL rats.

3.6. Impact of linagliptin treatment on BDL-induced cirrhotic rats

Table 3 depicts the hemodynamic and biochemistry parameters in the control (sham + vehicle), BDL (BDL + vehicle), and BDL + linagliptin groups. Rats in the BDL and BDL + linagliptin groups had a significantly lower BW compared with the control group (control vs BDL vs BDL + linagliptin: 492 ± 32 vs 374 ± 46 vs 401 ± 35 g; control vs BDL, BDL + linagliptin, $p < 0.05$). In addition, the MAP was significantly decreased and PP was significantly increased in the BDL and BDL + linagliptin groups compared with the control group (MAP, control vs BDL vs BDL + linagliptin: 152 ± 1 8 vs 131 ± 13 vs 120 ± 19 mmHg; control vs BDL, BDL + linagliptin, $p < 0.05$; PP, 8.8 ± 1.5 vs 15.9 ± 4.9 vs 14.1 ± 2.5 mmHg; control vs BDL, BDL + linagliptin, $p < 0.05$). SMAf was significantly increased in BDL rats compared with the control rats (SMAf, control vs BDL vs BDL + linagliptin: 4.2 ± 0.9 vs 5.9 ± 1.7 vs 5.1 ± 1.5 mL/min/100 g; control vs BDL, $p < 0.05$). The CI was also increased while the SVR was decreased in BDL rats compared with the control rats (CI, control vs BDL vs BDL + linagliptin: 32.8 ± 4.9 vs 45.6 ± 6.1 vs 40.5 ± 12.6 mL/min/100 g; control vs BDL, $p < 0.05$; SVR, 4.9 ± 0.8 vs 2.9 ± 0.5 vs 3.2 ± 0.9 mmHg/mL/min/100 g; control vs BDL, BDL + linagliptin, $p < 0.05$). However, linagliptin did not significantly alter the BW, MAP, PP, SMAf, CI, and SVR in BDL rats. BDL rats also had significantly elevated ammonia, ALT, AST, and total bilirubin levels compared with the control group, which were not significantly influenced by linagliptin (ammonia, control vs BDL vs BDL + linagliptin: 153 ± 36 vs 479 ± 252 vs 432 ± 132 μmol/L; control vs BDL, BDL + linagliptin, $p < 0.05$; ALT, 34 ± 6 vs 153 ± 69 vs 113 ± 47 IU/L; control vs BDL, BDL + linagliptin, $p < 0.05$; AST, 109 ± 19 vs 790 ± 280 vs 664 ± 253 IU/L; control vs BDL, BDL + linagliptin, $p < 0.05$; total bilirubin, 0.04 ± 0.02 vs 6.8 ± 1.7 vs 7.3 ± 1.2 mg/dL; control vs BDL, BDL + linagliptin, $p < 0.05$). The HR, PVf, creatinine, and fasting blood glucose levels were not significantly different among the control, BDL, and BDL + linagliptin groups.

3.7. mRNA expression in the splenorenal shunt and SMA in PVL rats

Fig. 3 shows the mRNA expression in the splenorenal shunt (the most prominent intra-abdominal collateral vessel) and the SMA of PVL rats (control vs linagliptin, n = 7:7). In the SMA of PVL rats, linagliptin significantly upregulated GLP-1 and eNOS mRNA expression (control vs linagliptin: GLP-1, 0.000014 ± 0.000007 vs 0.00089 ± 0.00075, $p = 0.009$; eNOS, 0.00045 ± 0.00017 vs 0.00110 ± 0.00055, $p = 0.01$). In the splenorenal shunt, eNOS mRNA expression was also significantly upregulated by linagliptin (control vs linagliptin: 0.000043 ± 0.000021

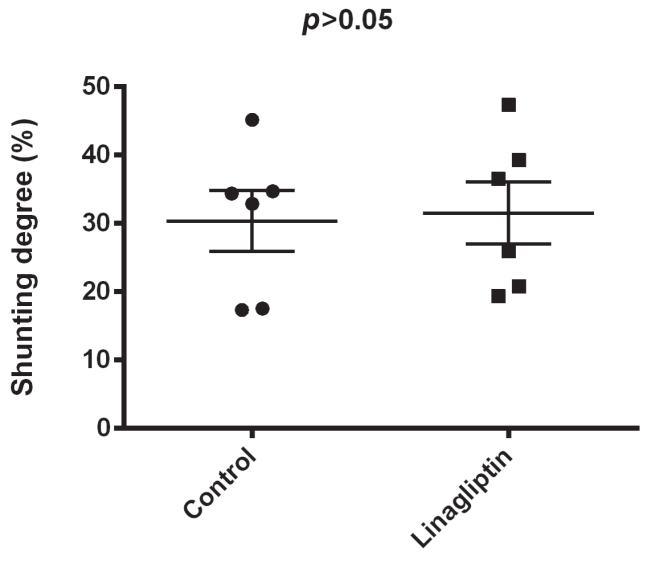

Fig. 1 Degree of portosystemic shunting in PVL rats treated with or without linagliptin. 10-Day linagliptin treatment did not affect the degree of shunting in PVL rats. PVL = portal vein ligation.

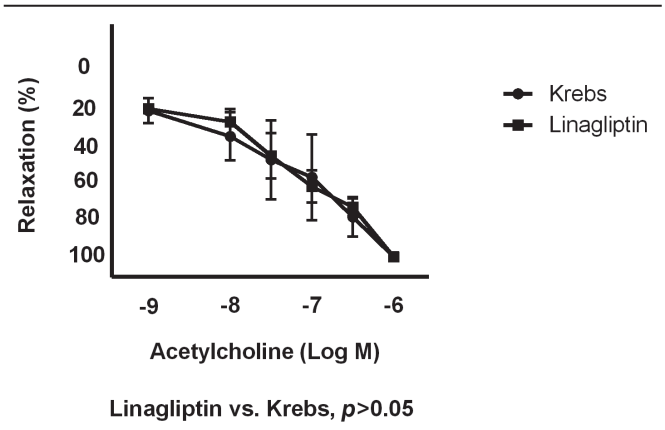

Fig. 2 Percentage of portosystemic collateral vascular relaxation to 10^{-9} to 10^{-6} M acetylcholine (100% relaxation in response to 10^{-6} M acetylcholine) in PVL rats after linagliptin or Krebs solution (control) preincubation. There was no significant difference between the linagliptin preincubated and control groups. PVL = portal vein ligation.

Table 3

BW, hemodynamic parameters, and biochemistry data of BDL rats with or without linagliptin (10 mg/kg) treatment

	Sham + vehicle (n = 7)	BDL + vehicle (n = 5)	BDL + linagliptin (n = 6)
BW (g)	492 ± 32	374 ± 46[a]	401 ± 35[a]
MAP (mmHg)	152 ± 18	131 ± 13[a]	120 ± 19[a]
PP (mmHg)	8.8 ± 1.5	15.9 ± 4.9[a]	14.1 ± 2.5[a]
HR (beats/min)	384 ± 40	371 ± 51	345 ± 21
PVf (mL/min/100 g)	7.8 ± 1.9	9.6 ± 2.2	7.5 ± 2.8
SMAf (mL/min/100 g)	4.2 ± 0.9	5.9 ± 1.7[a]	5.1 ± 1.5
SVR (mmHg/mL/min/100 g)	4.9 ± 0.8	2.9 ± 0.5[a]	3.2 ± 0.9[a]
CI (mL/min/100 g)	32.8 ± 4.9	45.6 ± 6.1[a]	40.5 ± 12.6
Ammonia (μmol/L)	153 ± 36	479 ± 252[a]	432 ± 132[a]
ALT (IU/L)	34 ± 6	153 ± 69[a]	113 ± 47[a]
AST (IU/L)	109 ± 19	790 ± 280[a]	664 ± 253[a]
TB (mg/dL)	0.04 ± 0.02	6.8 ± 1.7[a]	7.3 ± 1.2[a]
Cr (mg/dL)	0.47 ± 0.08	0.59 ± 0.20	0.64 ± 0.16
Glu (mg/dL)	118 ± 26	93 ± 31	89 ± 15

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BDL = bile duct ligation; BW = body weight; CI = cardiac index; Cr = creatinine; Glu = glucose; HR = heart rate; MAP = mean arterial pressure; PP = portal pressure; PVf = portal venous flow; SMAf = superior mesentery arterial flow; SVR = systemic vascular resistance; TB = total bilirubin.

[a] $p < 0.05$ compared with the sham + vehicle group.

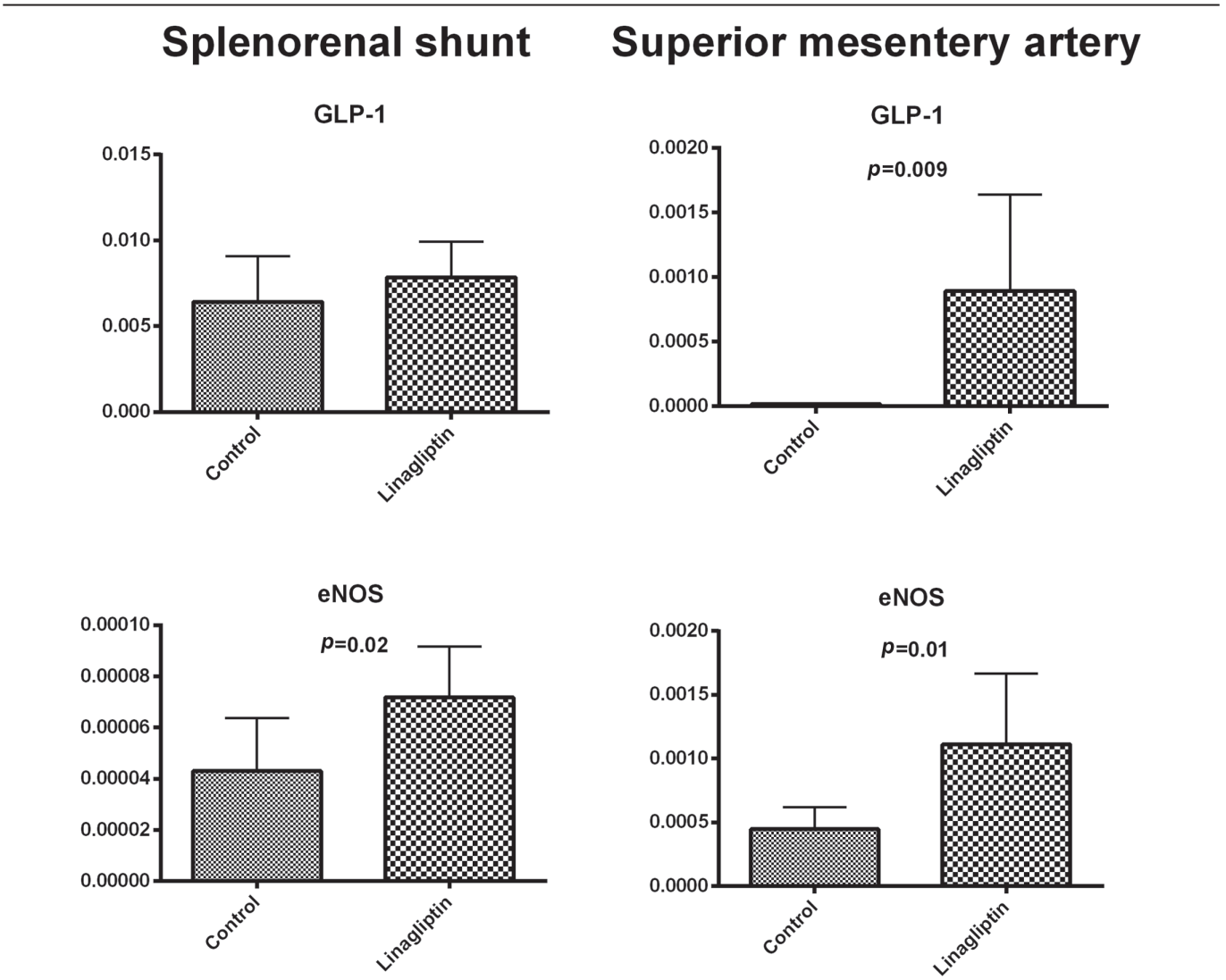

Fig. 3 Splenorenal shunt and SMA mRNA expression in PVL rats with or without linagliptin treatment. In the splenorenal shunt, eNOS mRNA expression was upregulated by linagliptin but GLP-1 mRNA expression was not affected. In the SMA, both eNOS and GLP-1 mRNA expression were significantly upregulated by linagliptin compared with the control group. eNOS = endothelial nitric oxide synthase; GLP-1 = glucagon-like peptide-1; PVL = portal vein ligation; SMA = superior mesentery artery.

vs 0.000072 ± 0.000020, p = 0.02) but GLP-1 mRNA expression was not significantly affected by linagliptin (control vs linagliptin: GLP-1, 0.0064 ± 0.0027 vs 0.0079 ± 0.0021; p > 0.05).

3.8. Hepatic protein expression of PVL and BDL rats with or without linagliptin treatment

Fig. 4 reveals the hepatic protein expression in PVL (n = 7:7) and BDL rats (n = 5:6) treated with linagliptin or the vehicle (control). In PVL rats, phosphorylated eNOS protein expression was significantly enhanced by linagliptin treatment (linagliptin vs control: 0.87 ± 0.31 vs 0.50 ± 0.06, p = 0.016). GLP-1 protein expression was not significantly influenced by linagliptin (0.82 ± 0.38 vs 0.60 ± 0.30; p > 0.05). Similarly, phosphorylated eNOS protein expression was significantly enhanced by linagliptin treatment in BDL rats (linagliptin vs control: 0.082 ± 0.021 vs 0.047 ± 0.025; p = 0.013). However, GLP-1 protein expression was not affected by linagliptin (0.034 ± 0.019 vs 0.041 ± 0.022; p > 0.05).

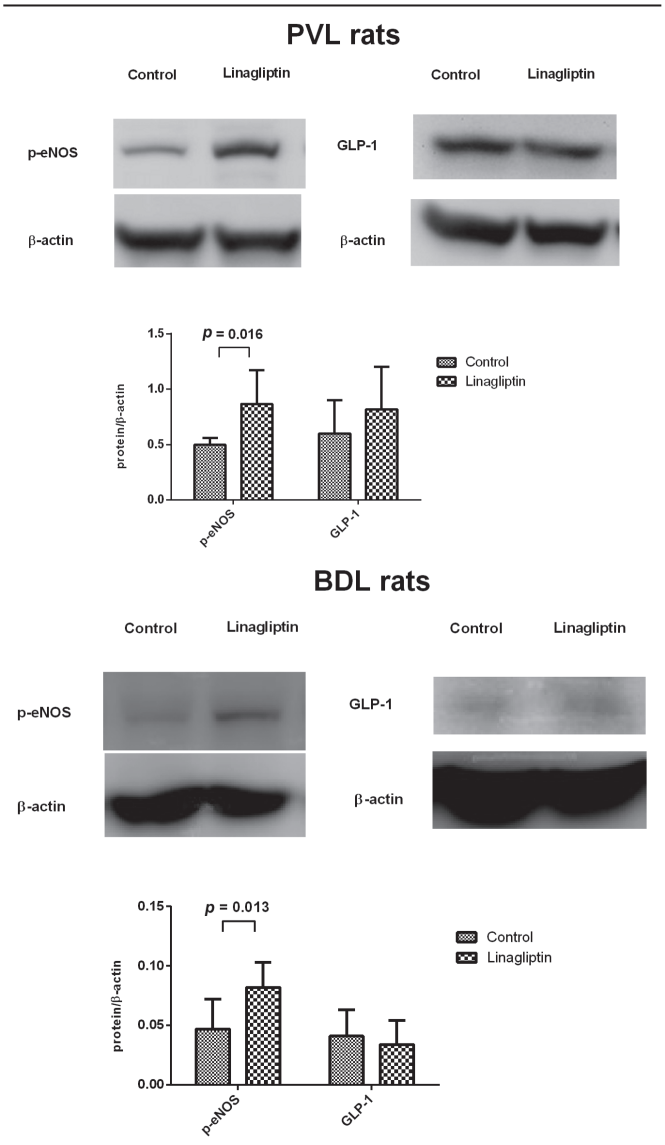

Fig. 4 Hepatic protein expression of linagliptin-treated PVL and BDL rats. The densitometric quantification and representative Western blot images are shown. Linagliptin treatment did not affect GLP-1 protein expression in PVL or BDL rats. However, phosphorylated-eNOS protein expression was upregulated by linagliptin in both PVL and BDL rats. BDL = bile duct ligation; eNOS = endothelial nitric oxide synthase; GLP-1 = glucagon-like peptide-1; PVL = portal vein ligation.

3.9. Histopathological changes in the liver of BDL rats

Fig. 5 depicts the histopathological changes in the hepatic tissue of sham-operated (control) and BDL rats with or without linagliptin treatment. Compared with the control rats, the livers of the BDL rats had increased mononuclear cell infiltration, ballooning changes in the hepatocytes and destruction of the lobular structure, indicating inflammatory changes. Sirius Red staining revealed obvious fibrosis of the liver in BDL rats. However, neither inflammation nor fibrosis were attenuated by linagliptin in the BDL rats.

4. DISCUSSION

The present study showed that DPP-4 inhibition by linagliptin reduced PP without significantly influencing systemic hemodynamics in rats with PVL-induced portal hypertension. PP is determined by three main factors: intrahepatic resistance, splanchnic blood flow as reflected by SMAf and PVf, and portal-systemic collateral vascular resistance. Since the portosystemic collateral vascular response (indicating vascular resistance), SMAf and PVf were not influenced by linagliptin, it is inferred that the linagliptin-induced PP reduction is mainly attributed to a reduction in intrahepatic resistance. Emerging evidence shows that DPP-4 plays a role in the development of vascular stiffness and endothelial dysfunction.[21] Inhibition of DPP-4 by linagliptin has also been reported to improve microvascular dysfunction in diabetic patients.[22] Furthermore, linagliptin significantly enhanced the vasodilatory response to acetylcholine in the mesentery artery of diabetic rats through enhancement of eNOS expression.[23] We consistently found that linagliptin upregulated SMA and collateral eNOS mRNA expression, as well as intrahepatic eNOS protein expression. Therefore, we postulated that linagliptin may reduce PP via NO-induced reduction of intrahepatic resistance. Interestingly, although linagliptin also upregulated eNOS expression at the SMA and splenorenal shunt, the splanchnic blood flow and collateral vasodilatory response to acetylcholine were not affected. Since it has been previously demonstrated that the splanchnic and collateral vascular beds have excessive NO production in portal hypertension,[4] the effect of linagliptin-induced eNOS upregulation may be negligible due to the existence of abundant NO in the splanchnic and collateral vasculature.

Similarly to PVL rats, linagliptin enhanced intrahepatic eNOS protein expression in BDL rats; however, it did not improve liver fibrosis and intrahepatic inflammation, which might explain the neutral portal-hypotensive effects of linagliptin in rats with BDL-induced cirrhosis. Enhanced eNOS-related vasodilatation could not completely reverse the increase in intrahepatic resistance of the fibrotic liver. A previous report showed that linagliptin significantly improved insulin sensitivity and lipid profile and reduced inflammatory mediators, and collagen depositions in diabetic rats with liver fibrosis.[24] Meanwhile, linagliptin treatments significantly improved liver fibrosis in carbon tetrachloride (CCl_4)–induced liver fibrosis.[14] Nevertheless, our data showed that linagliptin did not improve liver fibrosis in rats with BDL-induced biliary cirrhosis. This might be due to the different experimental models, since severe and relatively nonmodifiable liver cirrhosis has been established after BDL compared with CCl_4-induced liver fibrosis or diabetes mellitus related fatty liver.

Although PVL and BDL are both well-known animal models for portal hypertension, PVL rats had a lower CI and higher SVR and preserved liver function. In contrast, BDL rats had a higher CI and lower SVR accompanied by jaundice, suggesting

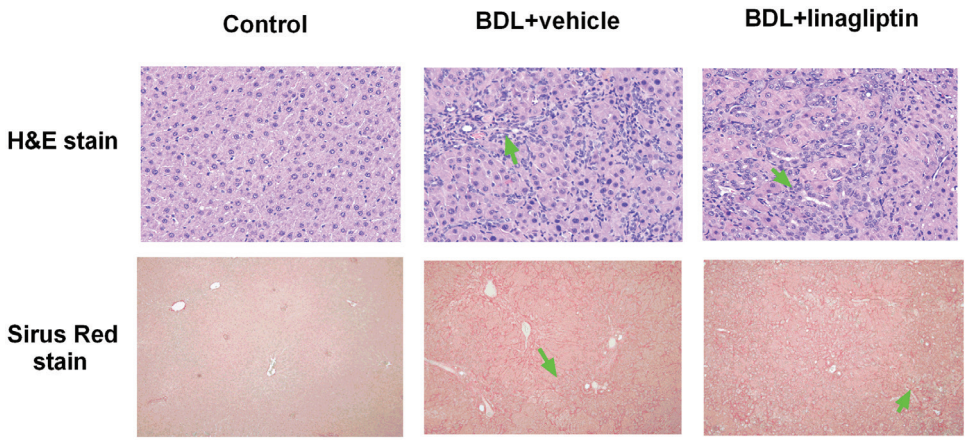

Fig. 5 Liver histology and Sirius Red stainings of sham or BDL rats treated by linagliptin or vehicle. Representative H&E staining images of BDL rats show ballooning changes in the hepatocytes accompanied by increased inflammatory cells (green arrow), indicating inflammatory changes in the liver (magnification ×200, upper panel). Liver fibrosis was demonstrated by Sirius Red staining (magnification ×40, green arrow indicating red area, lower panel). Compared with the control group, BDL rats had significantly increased liver fibrosis and inflammation. Linagliptin did not improve the hepatic inflammation and liver fibrosis in BDL rats. BDL = bile duct ligation; H&E = hematoxylin and eosin stain.

impaired liver function and histopathological changes of the liver. Taking the various degrees of liver injury and the tolerable doses of the two models into consideration, we chose different doses of linagliptin for each model. In addition, the initiation of linagliptin treatment in PVL rats started from the very beginning but in BDL rats it started from the 15th day post-BDL. Hence the different therapeutic effects of linagliptin in these two experimental models might be ascribed to the different dosages and duration of linagliptin administration.

Our data showed that linagliptin did not influence the blood glucose level or liver and renal biochemistry in portal hypertensive and cirrhotic rats. Therefore, linagliptin might have an acceptable safety profile for portal hypertensive and cirrhotic patients. Linagliptin is a highly selective DPP-4 inhibitor that is cleared mainly via the hepatobiliary mechanism.[25] It has been shown that patients with mild, moderate, and severe hepatic impairment do not exhibit an increase in linagliptin exposure after single and multiple dosages compared with those with normal hepatic function.[26] The safety of linagliptin use has also been previously documented in diabetic patients with liver disease.[27] A nondiabetic animal model was used in this study to exclude potential hyperglycemia- and glucose control-related pleiotropic effects, and our data showed that linagliptin did not induce hypoglycemia in portal hypertensive and cirrhotic rats. Therefore, the portal-hypotensive and pleiotropic properties of linagliptin are independent of its glucose-lowering effect.

DPP-4 inhibitors have been found to be beneficial for diabetes-related microangiopathy, wound healing, postmyocardial ischemic damage, and endothelial function in the vasculature of ischemic limbs.[28,29] DPP-4 inhibitors increase circulatory angiogenic cell numbers and ischemic limb blood flow in rats with critical limb ischemia, indicating its capacity to enhance angiogenesis.[30] The neoangiogenesis effect of DPP-4 inhibition raises some concerns regarding its promotion of portosystemic collateral vascular formation. However, our data revealed that 10-day linagliptin treatment did not significantly affect the degree of portosystemic shunting in portal hypertensive rats. Nevertheless, the impact of long-term linagliptin administration on gastroesophageal variceal formation or bleeding in a clinical setting should be evaluated.

The mechanism of DPP-4 inhibition on portal hypertension could be derived from both GLP-1–dependent and independent pathways. Liu et al[31] reported that DPP-4 inhibition by sitagliptin preserved vascular GLP-1/GLP-1 receptor function in spontaneous hypertensive rats, which subsequently induced eNOS activation, restored vascular relaxation, and increased renal blood flow. On the other hand, Kaji et al[14] demonstrated that DPP-4 inhibition attenuated hepatic fibrosis without altering intrahepatic GLP-1 mRNA expression. In the present study, we found that linagliptin significantly upregulated SMA GLP-1 mRNA expression without affecting hepatic GLP-1 protein expression in PVL rats. In addition, SMA, splenorenal shunt eNOS mRNA, and hepatic eNOS protein expression were upregulated. Since NO is an important vasodilator capable of reducing intrahepatic vascular resistance, our data suggest that the linagliptin-induced portal hypotensive effect was mediated, at least partly, through eNOS upregulation to reduce hepatic vascular resistance.

In conclusion, the current study shows that DPP-4 inhibition by linagliptin reduces portal pressure in an NO-dependent manner in portal hypertensive rats. A possible mechanism could be the decrease in intrahepatic resistance. However, the effect of linagliptin on cirrhotic rats is insignificant and warrants further investigation.

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