

# Dual angiotensin receptor and neprilysin inhibitor reduced portal pressure through peripheral vasodilatation and decreasing systemic arterial pressure in cirrhotic rats

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## Abstract

**Background:** Portal hypertension develops along with the progression of liver cirrhosis. Natriuretic peptides have been shown to reduce portal pressure but concomitantly activate the renin-angiotensin-aldosterone system (RAAS). Angiotensin receptor-neprilysin inhibitors (ARNIs) upregulate natriuretic peptides and avoid the adverse effects of RAAS activation. ARNIs have been shown to reduce portal pressure in rats with pre-hepatic portal hypertension, which involves relatively little liver injury. This study aimed to evaluate the relevant effects of an ARNI in rats with both liver cirrhosis and portal hypertension.

**Methods:** Male Sprague-Dawley rats received common bile duct ligation to induce liver cirrhosis and portal hypertension. Sham-operated rats served as surgical controls. All rats were randomly allocated into three groups to receive distilled water (vehicle), LCZ696 (an ARNI), or valsartan for 4 weeks. Portal hypertension and relevant derangements were assessed after treatment.

**Results:** Portal hypertension and hyperdynamic circulation developed in the cirrhotic rats. In the rats with cirrhosis and portal hypertension, both LCZ696 and valsartan reduced portal hypertension, mean arterial pressure, and systemic vascular resistance. The decrease in portal pressure was highly associated with the reduction in arterial pressure and systemic vascular resistance. Blood flow in hepatic, splanchnic, and portosystemic collateral systems was not altered. LCZ696 did not significantly influence liver injury or plasma cytokine levels. Liver fibrosis and splanchnic angiogenesis were not affected.

**Conclusion:** ARNI treatment exerted portal pressure lowering effects via peripheral vasodilatation and decreasing systemic arterial pressure in the rats with liver cirrhosis and portal hypertension. Caution should be taken when using ARNIs in liver cirrhosis.

**Keywords:** Angiogenesis; Angiotensin receptor-neprilysin inhibitor; Liver cirrhosis; Natriuretic peptide; Portal hypertension; Renin-angiotensin-aldosterone system

## 1. INTRODUCTION

Portal hypertension develops along with liver cirrhosis and results in lethal complications. The severity of portal hypertension has been shown to correlate with the prognosis of cirrhotic patients, and a

reduction in portal pressure has been shown to improve outcomes.<sup>1,2</sup> The renin-angiotensin-aldosterone system (RAAS) plays an important role in portal hypertension. In liver cirrhosis, angiotensin II is upregulated as a result of RAAS activation and increases portal pressure through the activation of stellate cells.<sup>3,4</sup> In addition, RAAS activation induces ascites and hepatorenal syndrome in liver cirrhosis.<sup>5</sup> However, the beneficial effects of RAAS inhibitors on portal hypertension are inconclusive in cirrhotic patients.<sup>6</sup> The major concern is that RAAS inhibition concomitantly induces systemic arterial hypotension, which may adversely affect the hemodynamic status of cirrhotic patients.<sup>7,8</sup> Therefore, RAAS inhibitors alone are still not recommended for the treatment of portal hypertension.

Angiotensin receptor-neprilysin inhibitors (ARNIs) are a new class of agent introduced for the treatment of heart failure, and they concomitantly modulate the RAAS and natriuretic peptides (NPs). The enzyme neprilysin catalyzes the degradation of NPs, and the inhibition of neprilysin results in the upregulation of NPs which exert antifibrotic, natriuretic and vasodilatation effects.<sup>9</sup> A previous study showed that an NP analogue could reduce portal pressure by enhancing splanchnic vasoconstriction.<sup>10</sup> In addition, neprilysin inhibitor treatment has been shown to attenuated

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portal hypertension via reducing intrahepatic vascular resistance.<sup>11</sup> Moreover, the inhibition of neprilysin can activate the RAAS. Therefore, the combination of a neprilysin inhibitor and RAAS inhibitor has become a feasible way to activate NPs and avoid the adverse effects of RAAS activation at the same time.

Our previous study showed that LCZ696, an ARNI composed of sacubitril and valsartan, could effectively ameliorate portal hypertension in portal hypertensive rats via downregulation of hepatic vasoconstrictors.<sup>12</sup> In that study, portal hypertension was induced by partial portal vein ligation, which is an animal model with pre-hepatic portal hypertension but without significant liver pathological change that the effects of ARNI on liver cirrhosis is still unknown. Therefore, we aimed to evaluate the effects of an ARNI in rats with both liver cirrhosis and portal hypertension.

## 2. METHODS

### 2.1. Animal model

Male Sprague-Dawley rats weighing 240-270 g at the time of surgery were used for the experiments. The rats were housed in plastic cages and allowed free access to food and water. All rats were fasted for 12 hours before the operation. Secondary biliary cirrhosis was induced using common bile duct ligation (BDL).<sup>13</sup> Under ketamine anesthesia (100 mg/kg, intramuscularly), the common bile duct was doubly ligated with 3-0 silk. The first ligature was made below the junction of the hepatic ducts, and the second ligature was made above the entrance of the pancreatic duct, followed by sectioning of the common bile duct between the ligatures. The rats were allowed to recover afterward. A high yield of secondary biliary cirrhosis was noted 4 weeks after the ligation. To avoid coagulation defects, the BDL rats received weekly intramuscular vitamin K injection (50 µg/kg).

This study was approved by Taipei Veterans General Hospital Animal Committee (IACUC 2021-141). All experimental procedures were conducted in accordance with the standard procedures indicated in the principles of laboratory animal care (Guide for the Care and Use of Laboratory Animals, DHEW publication No. [NIH] 85-23, rev. 985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD, USA).

### 2.2. Study design

BDL was used to induce chronic liver injury and cirrhosis. Sham-operated rats served as surgical controls. BDL or sham rats were randomly allocated to receive one of the three following treatments: vehicle, LCZ696 (an ARNI; 40 mg/kg/d, oral gavage), or valsartan (20.8 mg/kg per day, equal to the amount of valsartan in LCZ696)<sup>14</sup> from the first day to the 28th day after BDL. Experiments were performed on the 29th day.

### 2.3. Measurement of systemic and portal hemodynamics

The right carotid artery was cannulated with a PE-50 catheter that was connected to a pressure transducer. Continuous recordings of mean arterial pressure (MAP), heart rate (HR), and portal pressure (PP) were performed on a multichannel recorder (MP45, Biopac Systems Inc., Goleta, CA, USA). The external zero reference was placed at the level of the mid-portion of the rat. The abdomen was then opened with a mid-line incision, and the mesenteric vein was cannulated with a PE-50 catheter connected to the transducer.<sup>15</sup>

The superior mesenteric artery (SMA) was identified at its aortic origin, and a 5-mm segment was gently dissected free from surrounding tissues. Then a pulsed-Doppler flow transducer (TS420, Transonic System Inc., Ithaca, NY, USA) was placed to measure the SMA flow.<sup>16</sup> Hepatic inflow via the portal vein (portal part) was also measured by placing a flow probe around the portal vein

as proximal to the liver as possible. Splenorenal shunt (SRS) flow was measured with a flow probe around the SRS.<sup>17</sup>

Cardiac output was measured by thermodilution, as previously described.<sup>18</sup> Briefly, a thermistor was placed in the aortic arch just distal to the aortic valve, and the thermal indicator (100 µL of normal saline) was injected into the right atrium through a PE-50 catheter. The aortic thermistor was connected to a cardiac output computer Cardiomax III (Columbus Instruments International Co., Columbus, OH, USA). Five thermodilution curves were obtained for each cardiac output measurement. The final value was obtained from the arithmetic mean of the computer results. Cardiac index (mL/min/100 g body weight [BW]) was calculated as cardiac output per 100 g BW. Systemic vascular resistance (SVR, mmHg/mL/min/100 g BW) was calculated by dividing the MAP by the cardiac index. SMA resistance (mmHg/mL/min/100 g BW) was calculated as (MAP-PP)/SMA flow per 100 g BW. Hepatic vascular resistance (mmHg/mL/min/100 g BW) was calculated as PP/hepatic inflow (portal part) per 100 g BW.

### 2.4. Immunofluorescent study for mesenteric vascular density

Mesenteric angiogenesis was quantified using CD31-labeled microvascular networks in rat mesenteric connective tissue windows according to a previous study.<sup>16</sup> At least four mesenteric windows (wedge-shaped regions of connective tissue bordered by the intestinal wall and ileal blood vessel pairs) were dissected free from each rat, washed in PBS, dried on gelatin slides, and fixed in 100% MeOH (-20°C for 30 minutes). The slides were then incubated overnight at 4°C with the primary antibody mouse anti-rat CD31-biotin (AbD Serotec, Oxford, United Kingdom). The secondary antibody (CY2-conjugated streptavidin; Jackson ImmunoResearch, West Grove, PA, USA) was then applied for 1 hour at room temperature. At least four sets of data were obtained for each mesenteric window, and ( $\times 100$ )-magnification immunofluorescent images were assessed using an upright fluorescent microscope (AX80, Olympus, Tokyo, Japan) and thresholded using Image J software (<http://rsb.info.nih.gov/ij/>). The vascular length was manually measured using the pencil tool, and the vascular area was measured automatically using the histogram function.

### 2.5. Hepatic fibrosis determination with Sirius red staining

Liver paraffin sections were stained with Sirius red (Polysciences Inc., Warrington, PA, USA). Image J was used to measure the percentage of Sirius red-stained area. Briefly, grayscale images were used, and the red-stained collagen was isolated using the thresholding function. The thresholded area was then measured and shown as the percentage of thresholded area per image.<sup>16</sup>

### 2.6. Drugs

LCZ696 and valsartan were purchased from Novartis International AG (Basel, Switzerland). All solutions were freshly prepared on the day of the experiment.

### 2.7. Statistical analysis

All results are expressed as mean  $\pm$  SEM. Statistical analyses were performed using an unpaired Student's *t* test or one-way analysis of variance (ANOVA) as appropriate. Least Significant Difference (LSD) was used for the post hoc test. SPSS version 21 for Windows (SPSS Inc., Chicago, IL, USA) was used for all analyses. Results were considered statistically significant at two-tailed *p* values of less than 0.05.

## 3. RESULTS

The rats underwent BDL to induce liver cirrhosis and portal hypertension. Compared with the sham-operated control rats,

**Table 1**

**Hemodynamic parameters in rats with sham operation (control) or cirrhosis induced by common BDL**

Hemodynamic parameters	Control (n = 6)	BDL (n = 8)
BW (g)	460 ± 14	403 ± 11 <sup>b</sup>
PP (mmHg)	8.3 ± 0.4	16.8 ± 0.7 <sup>c</sup>
Systemic circulation		
MAP (mmHg)	148 ± 5	131 ± 6
HR (beats/min)	380 ± 15	379 ± 6
CI (mL/min/100 g)	35.9 ± 1.5	41.4 ± 2.9
SVR (mmHg/mL/min/100 g)	4.2 ± 0.2	3.3 ± 0.3 <sup>a</sup>
Splanchnic system		
SMA flow (mL/min/100 g)	4.3 ± 0.2	5.7 ± 0.3 <sup>b</sup>
Portal system		
Hepatic inflow (portal part) (mL/min/100 g)	5.6 ± 0.3	11.3 ± 0.4 <sup>c</sup>
Collateral system		
SRS flow (mL/min/100 g)	0.04 ± 0.01	1.58 ± 0.28 <sup>c</sup>

BDL = bile duct ligation; BW = body weight; CI = cardiac index; HR = heart rate; MAP = mean arterial pressure; PP = portal pressure; SMA = superior mesenteric artery; SRS = splenorenal shunt; SVR = systemic vascular resistance.

<sup>a</sup>*p* < 0.05.

<sup>b</sup>*p* < 0.01.

<sup>c</sup>*p* < 0.001.

the cirrhotic rats had significant portal hypertension (Table 1). The BDL rats had a significantly lower BW. The hemodynamic study revealed that the cirrhotic rats had lower SVR and higher SMA flow, hepatic flow, and SRS flow. These results suggested hyperdynamic circulation and prominent portosystemic collateral shunts in the BDL rats.

The results of plasma analysis are shown in Table 2. In the BDL group, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin concentrations were significantly increased. The concentration of plasma interleukin (IL)-1 $\beta$  tended to be higher, and the concentration of tumor necrosis factor (TNF)- $\alpha$  was significantly higher in the BDL group. These data indicated cholestasis, liver injury, and systemic inflammation in the cirrhotic rats. Taken together, the BDL rats had the typical presentation of portal hypertension.

### 3.1. Effects of LCZ696 and valsartan on the sham-operated control rats

The sham-operated control rats received distilled water as vehicle control (SD), LCZ696 (SL), or valsartan (SV) for 4 weeks (Fig. 1A). The effects of treatments were then evaluated. The results showed that neither LCZ696 nor valsartan influenced the PP in the rats without portal hypertension (Fig. 1B). Both LCZ696 and valsartan effectively reduced MAP (Fig. 1C;

148 ± 5 vs 116 ± 9 vs 122 ± 9 mmHg, SD vs SE, *p* = 0.011; SD vs SV, *p* = 0.028). There were no significant differences in AST, ALT, or total bilirubin among the three groups (Fig. 1D). The structural components inclusive of liver fibrosis and extrahepatic angiogenesis were evaluated by liver Sirius red staining and mesenteric immunofluorescent study, respectively (Fig. 2). The results showed that the severity of liver fibrosis and extrahepatic angiogenesis were not affected by LCZ696 or valsartan. Taken together, neither LCZ696 nor valsartan affected liver injury or portal hypertension-related hemodynamic status, except for a blood pressure lowering effect.

### 3.2. Effects of LCZ696 and valsartan on the cirrhotic rats

One day after BDL, the rats received distilled water as control (BD), LCZ696 (BL), or valsartan (BV) for 4 weeks (Fig. 3A). The effects of LCZ696 and valsartan on cirrhosis and portal hypertension were then evaluated. The results showed that both LCZ696 and valsartan significantly attenuated portal hypertension (Fig. 3B; 16.8 ± 0.7 vs 13.3 ± 1.7 vs 12.8 ± 0.8 mmHg, BD vs BL, *p* = 0.004; BD vs BV, *p* = 0.001). However, MAP was markedly decreased in the LCZ696 and valsartan groups compared with the control group (131 ± 6 vs 94 ± 7 vs 81 ± 8 mmHg, BD vs BL, *p* = 0.001; BD vs BV, *p* < 0.001) (Fig. 3C). SVR was also significantly decreased, while cardiac index was not affected (SVR: 3.3 ± 0.3 vs 2.3 ± 0.2 vs 1.9 ± 0.2 mmHg/mL/min/100 g, BD vs BL, *p* = 0.007; BD vs BV, *p* = 0.001). Blood flow in hepatic, splanchnic, or portosystemic collateral systems was not affected (Fig. 3D). These results suggested that both LCZ696 and valsartan induced peripheral vasodilatation and thus reduced MAP, which may, at least in part, contribute to the portal hypotensive effect of LCZ696 and valsartan.

LCZ696 and valsartan significantly increased plasma renin activity and attenuated plasma aldosterone level (plasma renin activity: 20.2 ± 5.7 vs 49.9 ± 10.6 vs 60.4 ± 7.9 mU/mL, BD vs BL, *p* = 0.05; BD vs BV, *p* = 0.006); Plasma aldosterone level: 724 ± 31.2 vs 536 ± 61.9 vs 525 ± 31.0 pg/mL, BD vs BL, *p* = 0.015; BD vs BV, *p* = 0.008) (Fig. 4A). The results confirmed that LCZ696 and valsartan had enough effect on RAAS. On the other hand, neither LCZ696 nor valsartan affected liver injury or systemic inflammation (Fig. 4B). In addition, plasma AST, ALT, and total bilirubin were not affected by the treatment. There was no significant difference in plasma IL-1 $\beta$  or TNF- $\alpha$  concentration among three groups (Fig. 4C). Moreover, neither LCZ696 nor valsartan influenced the severity of liver fibrosis (Fig. 5; 24.2% ± 0.9% vs 25.8% ± 1.1% vs 26.1% ± 0.9%, BD vs BL, *p* = 0.268; BD vs BV, *p* = 0.168). Mesenteric vascular density was not affected by the two treatments. Protein expression in liver and mesentery was evaluated (Supplementary Fig. 1, <http://links.lww.com/JCMA/A198>). The results showed that endothelial nitric oxide synthase (eNOS) and phospho-eNOS were upregulated in the liver of LCZ696 or valsartan treated groups. There was no significant difference among the three groups in the liver. The expressions were consistent in the mesentery. However, hepatic flow and splanchnic flow was not affected by LCZ696 or valsartan. Taken together, the effects of LCZ696 or valsartan on hepatic or splanchnic system was not significant enough to reverse the hemodynamic derangements.

## 4. DISCUSSION

In this study, we evaluated the effects of an ARNI in rats with cirrhosis and portal hypertension. Consistent with a previous study of portal hypertensive rats, LCZ696 ameliorated portal hypertension.<sup>11</sup> However, LCZ696 did not affect blood flow in hepatic, splanchnic, or portosystemic collateral systems in the rats with liver cirrhosis. In addition, liver fibrosis was not

**Table 2**

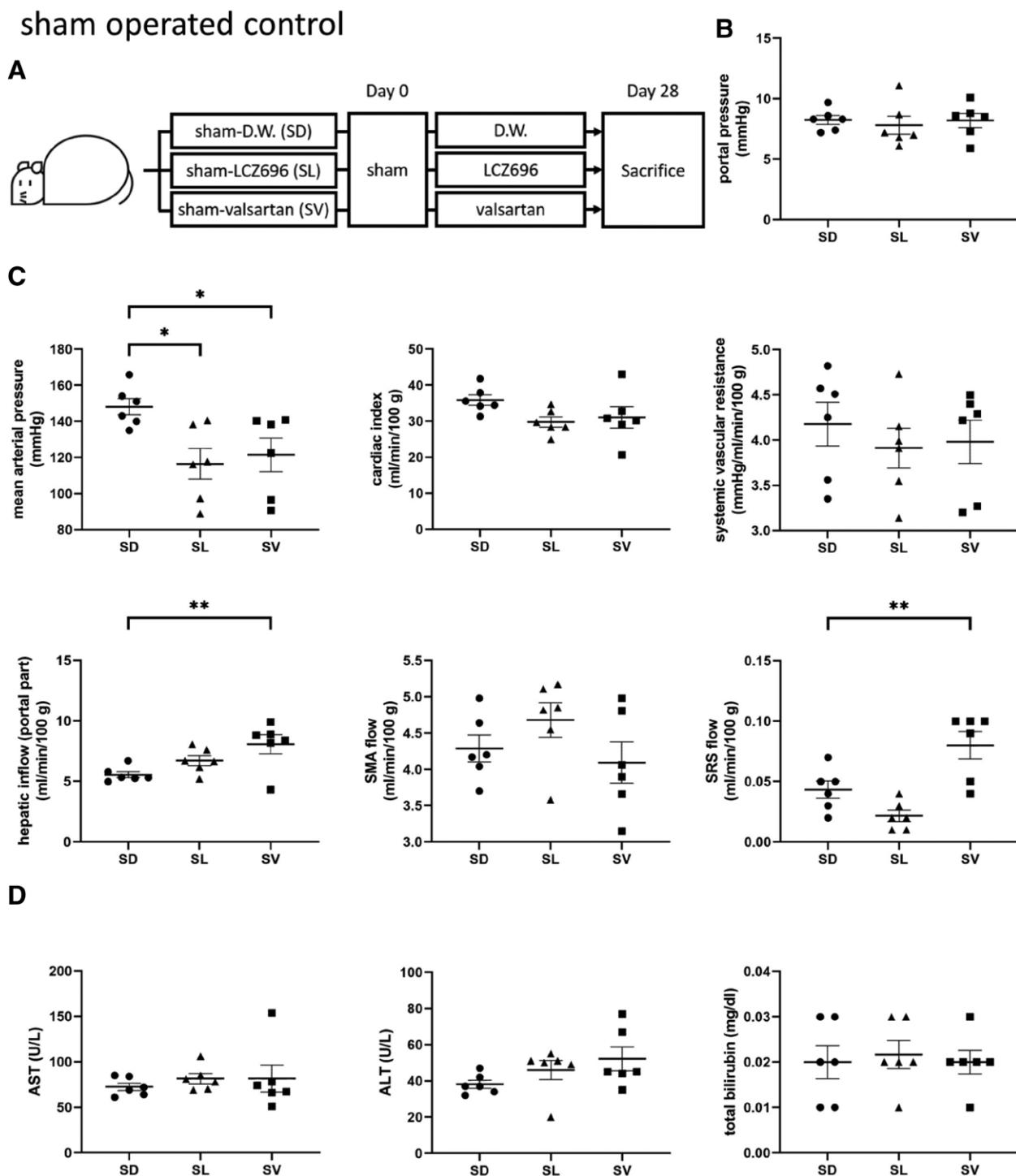
**Plasma biochemistry markers and cytokine levels in the rats with sham operation (control) or cirrhosis induced by common BDL**

Plasma biochemistry markers	Control (n = 6)	BDL (n = 8)
AST (U/L)	73 ± 4	672 ± 58 <sup>b</sup>
ALT (U/L)	38 ± 2	124 ± 11 <sup>b</sup>
Total bilirubin (mg/dL)	0.02 ± 0.00	8.66 ± 0.62 <sup>b</sup>
IL-1 $\beta$ (pg/mL)	18.2 ± 0.4	29.1 ± 5.9
TNF- $\alpha$ (pg/mL)	7.7 ± 0.5	21.6 ± 3.0 <sup>a</sup>

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BDL = bile duct ligation; IL = interleukin; TNF = tumor necrosis factor.

<sup>a</sup>*p* < 0.01.

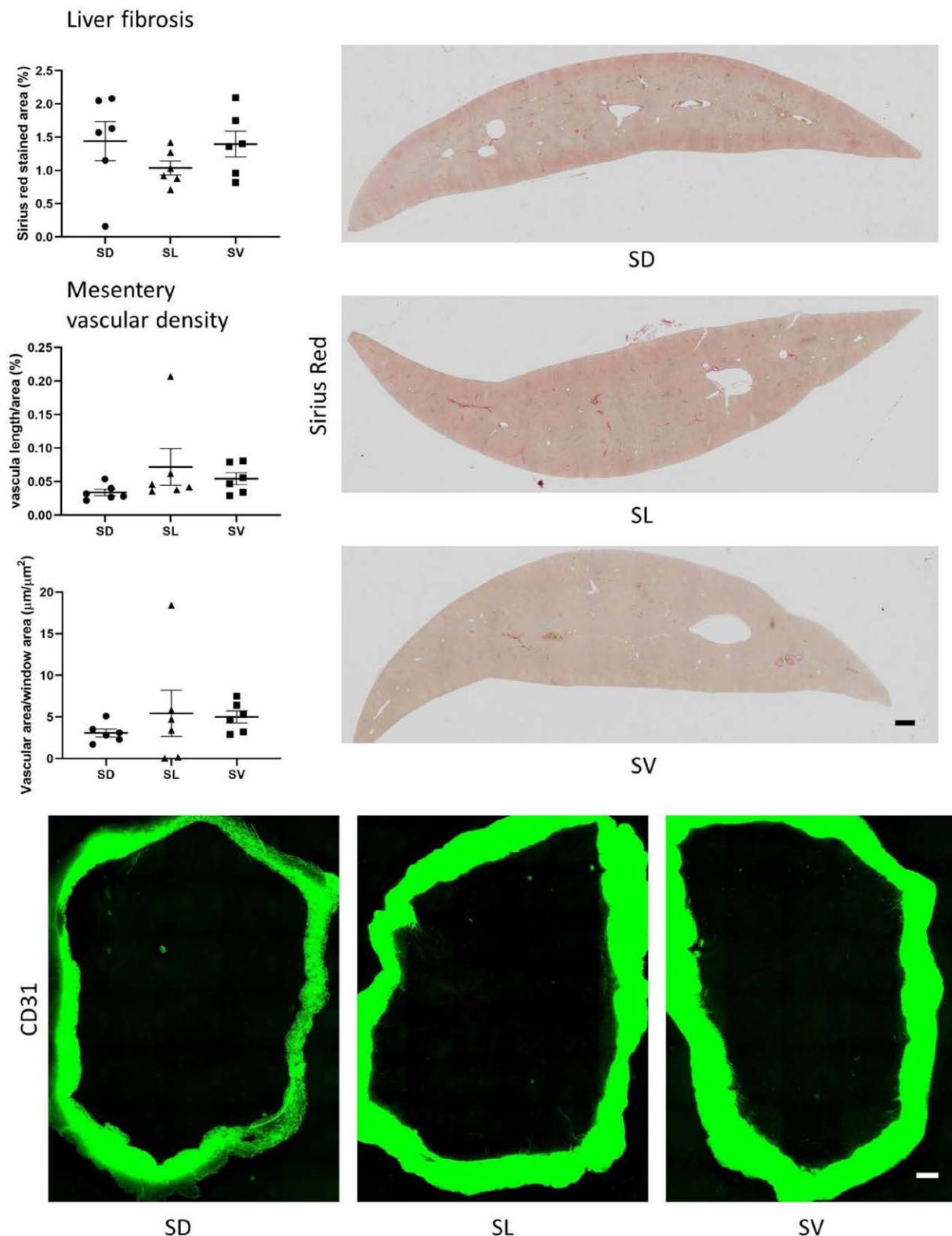
<sup>b</sup>*p* < 0.001.



**Fig. 1** Treatment effects in the sham-operated control rats. A, Sham-operated control rats received distilled water as control (SD), LCZ696 (SL), or valsartan (SV) for 4 wk (n = 6 in each group). B, Neither LCZ696 nor valsartan influenced portal pressure. C, LCZ696 and valsartan effectively reduced the mean arterial pressure. D, There were no significant differences in AST, ALT, or total bilirubin levels among the three groups. \*p < 0.05, \*\*p < 0.01. ALT = alanine aminotransferase; AST = aspartate aminotransferase; SMA = superior mesenteric artery; SRS = splenorenal shunt.

affected by LCZ696. On the other hand, SVR and MAP were significantly decreased in the LCZ696-treated cirrhotic rats. Taken together, ARNI<sub>s</sub> may exert a PP lowering effect by, at least in part, decreasing systemic blood pressure.

Increased hepatic vascular resistance is a major factor in the development and maintenance of portal hypertension. In the cirrhotic liver, regenerative nodules and collagen fiber deposition increase intrahepatic resistance, which serves as the structural component.<sup>19</sup> The RAAS enhances both inflammation and

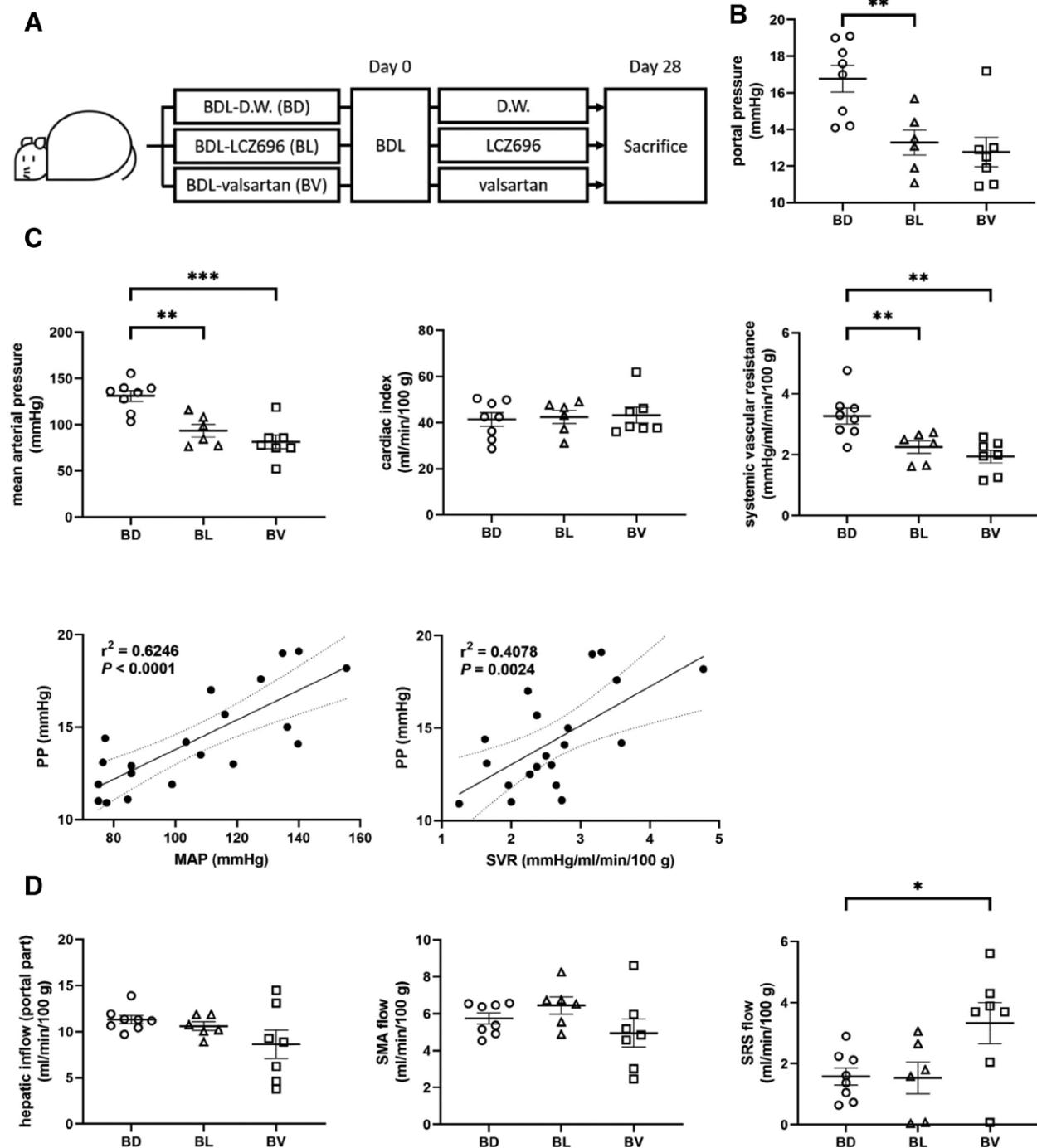


**Fig. 2** Hepatic and extra-hepatic structural changes in the sham-operated rats with different treatment. A, Liver fibrosis was evaluated by Sirius red staining. Liver fibrosis severity was not affected by LCZ696 or valsartan. Scale bar = 1 mm. B, Extrahepatic angiogenesis was assessed by mesenteric immunofluorescent study. There were no significant differences in vascular area ratio or vascular length among three groups. SD = sham-distilled water; SL = sham-LCZ696; SV = sham-valsartan.

fibrogenesis. Angiotensin II has been shown to increase renal TNF- $\alpha$  production and facilitate fibrotic processes.<sup>20</sup> In addition, ARNIs have been shown to ameliorate renal fibrosis and

inflammation in dogs.<sup>21</sup> Atrial natriuretic peptide was also shown to attenuate liver injury and reduce TNF- $\alpha$  in an ischemia and reperfusion model.<sup>22</sup> In addition, angiotensin-converting

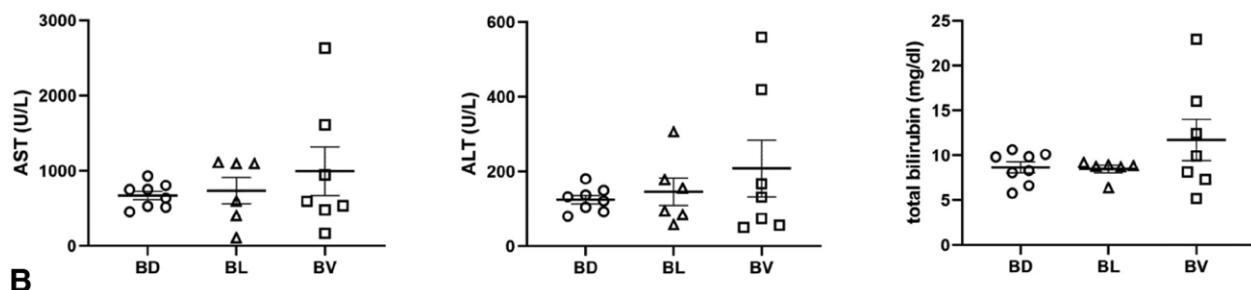
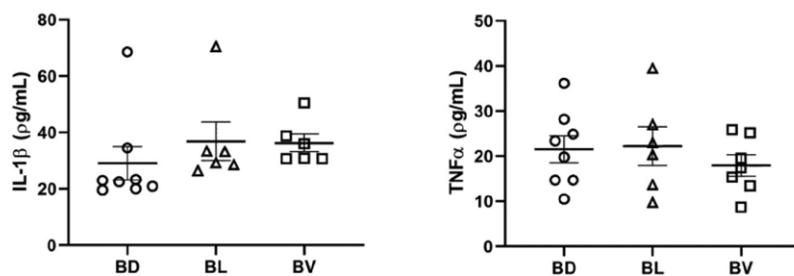
## Cirrhosis



**Fig. 3** Treatment effects in the cirrhotic rats. A, Rats received common bile duct ligation (BDL) to induce liver cirrhosis and portal hypertension. They were randomly allocated into three groups to receive distilled water as vehicle control (BD), LCZ696 (BL), or valsartan (BV) for 4 wk ( $n = 8, 6, 7$ ). B, Both LCZ696 and valsartan significantly attenuated portal hypertension compared with the control group. There was no significant difference between the BL and BV groups. C, MAP and SVR markedly decreased in the LCZ696 and valsartan groups compared with the control group, while CI was not affected. PP was significantly correlated with MAP ( $p < 0.0001$ ) and SVR ( $p = 0.0024$ ). D, Blood flow in hepatic, splanchnic, and portosystemic collateral systems was not affected. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . CI = cardiac index; MAP = mean arterial pressure; PP = portal pressure; SMA = superior mesenteric artery; SRS = splenorenal shunt; SVR = systemic vascular resistance.

enzyme inhibitors (ACEIs) and neutral endopeptidase (NEP) inhibitors have been shown to work synergistically in the reduction of cardiac pro-collagen synthesis.<sup>23</sup> In our previous study, we found that ARNI treatment reduced liver injury in rats with pre-hepatic portal hypertension. In addition, both ALT and AST

decreased in the valsartan and LCZ696-treated groups compared with the control group. However, in this study, LCZ696 did not affect liver enzyme or plasma cytokine concentrations in the cirrhotic rats. Moreover, the severity of liver fibrosis was not attenuated by LCZ696 either, and hepatic vascular resistance

**A****B**

**Fig. 4** Liver injury and systemic inflammation in the cirrhotic rats with different treatment. A, Plasma renin activity significantly increased and plasma aldosterone level significantly decreased. B, Plasma AST, ALT, and total bilirubin levels were not significantly different among the three groups. C, Plasma IL-1 $\beta$  and TNF- $\alpha$  levels were not significantly different among the three groups. ALT = alanine aminotransferase; AST = aspartate aminotransferase; BD = bile duct ligation-distilled water; BL = bile duct ligation-LCZ696; BV = bile duct ligation-valsartan; IL = interleukin; TNF = tumor necrosis factor.

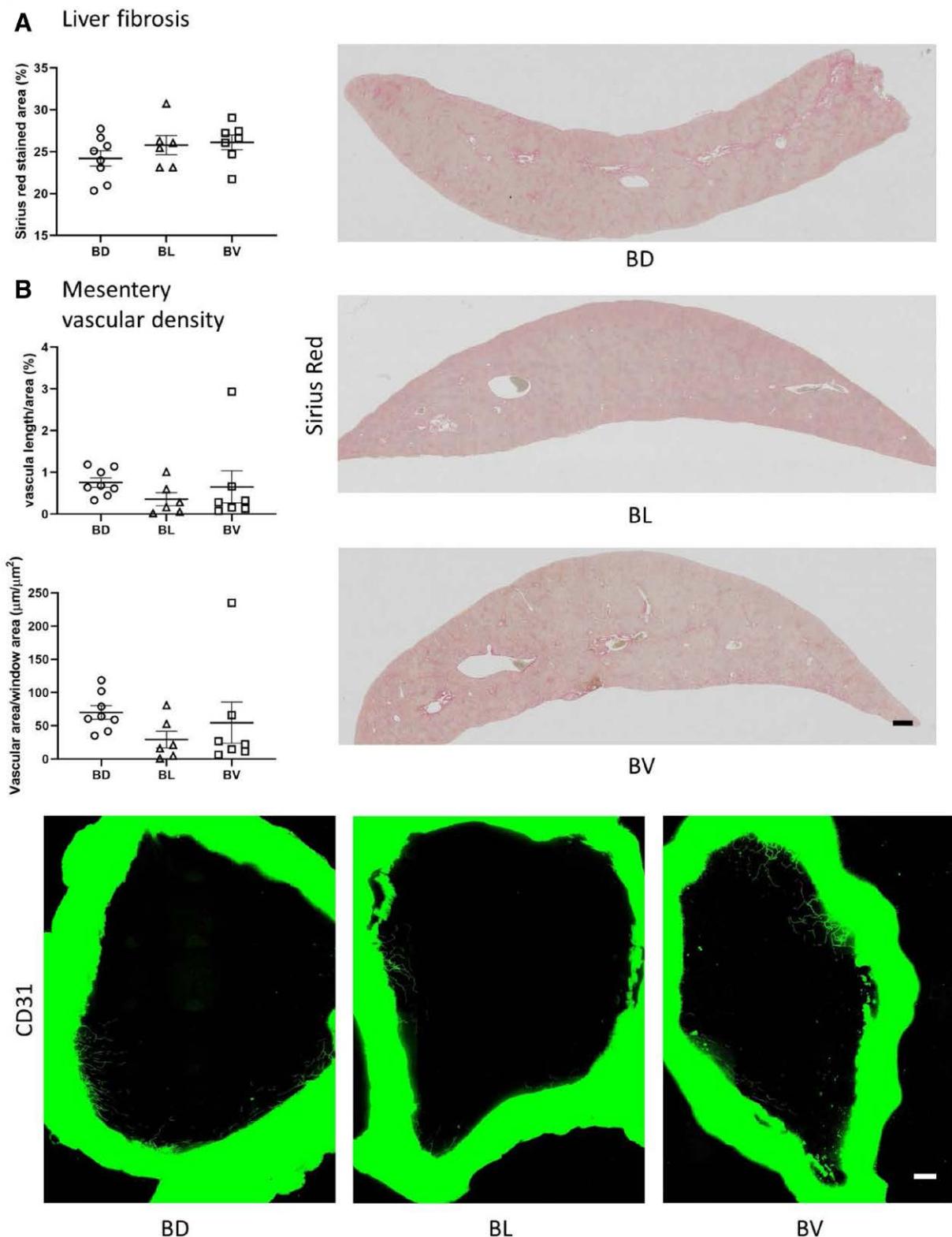
was not significantly different among the control, valsartan and LCZ696 groups. The discrepancy between the two studies may be due to different animal models. Rats with partial portal vein ligation have relatively mild liver injury, which may render it easier to be improved. In contrast, BDL results in severe liver injury and cirrhosis within 4 weeks, which may be too overwhelming for ARNI to overcome. In summary, ARNIs may have liver protective effects in certain conditions, but they were not detected in this study with an animal model of cholestatic liver cirrhosis.

In addition to liver fibrosis, functional changes in hepatic sinusoidal endothelial cells and hepatic stellate cells result in overt vasoconstriction and elevated PP. Angiotensin II increases the PP by enhancing the adrenergic effect and stellate cell constriction.<sup>4</sup> In liver cirrhosis, angiotensin II is upregulated as the result of RAAS activation, which then further aggravates portal hypertension.<sup>3,24</sup> Previous study showed that losartan, an angiotensin II receptor blocker (ARB), attenuated portal hypertension in cirrhotic rats.<sup>25</sup> On the other hand, losartan has been shown to elicit a significant reduction in hepatic venous pressure gradient, a clinical index of PP in cirrhotic patients.<sup>26</sup> Moreover, Arroyo et al<sup>27</sup> reported that ARB treatment significantly decreased wedged hepatic venous pressure but did not affect hepatic flow. In addition, the decrease in wedged hepatic venous pressure was associated with a reduction in arterial pressure. These results suggest that the inhibition of angiotensin II can reduce post-sinusoidal hepatic vascular resistance. In this study, ARNI treatment decreased the PP without influencing hepatic blood flow from the portal site. This is consistent with the effects of ARBs in cirrhotic patients. Taken together, these findings suggest that ARNIs do not affect functional components in the liver.

Splanchnic angiogenesis also plays an important role in portal hypertension. ARBs exert anti-angiogenesis activity, and valsartan was shown to markedly decrease capillary density

in hamsters with cardiomyopathy.<sup>28</sup> In addition, in rats with steatohepatitis, ARB treatment has been shown to significantly decrease the formation of new vessels both in vitro and in vivo.<sup>29,30</sup> On the other hand, the effects of NEP on angiogenesis are not well known. In this study, we found that treatment with an ARNI did not significantly affect splanchnic angiogenesis in the cirrhotic rats. The result was different to aforementioned studies due to different animal models.

Clinical studies have demonstrated the effects of ACEIs/ARBs on portal hypertension. A meta-analysis of 19 controlled trials and more than 650 patients<sup>6</sup> revealed that ACEIs/ARBs had comparable PP lowering effects to propranolol. However, liver cirrhosis is accompanied by hyperdynamic circulation, which is characterized by peripheral vasodilation, reduced MAP, and increased cardiac output, and if homeostasis of systemic perfusion cannot be maintained, organ injury may occur. Clinical studies have shown that inhibition of the RAAS resulted in arterial hypotension.<sup>7,8</sup> Therefore, RAAS inhibitors alone are not recommended for the treatment of portal hypertension. On the other hand, a previous study found that aladotrilat (a mixed ACE/NEP inhibitor) and captopril (a selective ACEI) slightly reduced the MAP in conscious rats with myocardial infarction.<sup>31</sup> Similar findings were reported in young (9-week-old) spontaneous hypertensive rats,<sup>32</sup> supporting that dual NEP/ACE inhibition did not have an additional hypotensive effect over selective ACE inhibition. In a human study, ARNI treatment induced hypotension in only 2.7% of patients with severe heart failure.<sup>33</sup> In our previous study, LCZ696 attenuated portal hypertension. It also reduced MAP through peripheral vasodilatation. In this study, we also found that ARNI treatment ameliorated portal hypertension accompanied by a reduction in MAP. Since maintaining systemic hemodynamic homeostasis and organ perfusion is very important in liver cirrhosis, this remains a major concern in applying ARNIs to cirrhotic patients.



**Fig. 5** Hepatic and extra-hepatic structural changes in the cirrhotic rats with different treatment. A, Neither LCZ696 nor valsartan significantly influenced the severity of liver fibrosis. B, Neither LCZ696 nor valsartan significantly influenced the mesenteric vascular density. BD = bile duct ligation-distilled water; BL = bile duct ligation-LCZ696; BV = bile duct ligation-valsartan.

In conclusion, treatment with an ARNI ameliorated portal hypertension without affecting liver inflammation, fibrosis or splanchnic angiogenesis in this study. However, the concomitant

peripheral vasodilatation and systemic hypotension are important factors, and hypotension cautions us against applying ARNI in liver cirrhosis.

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## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at <http://links.lww.com/JCMA/A198>.

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