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# Carvedilol Ameliorates Intrahepatic Angiogenesis, Sinusoidal Remodeling and Portal Pressure in Cirrhotic Rats

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Data Interpretation D  
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**Background:** Carvedilol is the first-line drug for the primary prophylaxis of variceal bleeding due to portal hypertension (PHT) in liver cirrhosis. This study aimed to investigate the effects of carvedilol on intrahepatic angiogenesis and sinusoidal remodeling in cirrhotic rats and explore the underlying mechanisms of carvedilol in PHT.

**Materials/Methods:** For *in vivo* experiments, carbon tetrachloride was used to induce liver cirrhosis in rats, and carvedilol was simultaneously administered by gavage. The portal pressure was measured in rats, and liver tissues were examined by immunohistochemistry. Sinusoidal remodeling was observed by transmission electron microscopy. For *in vitro* experiments, the effects of carvedilol on fibronectin (FN) synthesis in human umbilical vein endothelial cells (HUVECs) were explored by quantitative real-time polymerase chain reaction and western blot analysis.

**Results:** Portal vein pressure measurements showed that carvedilol reduced portal pressure in cirrhotic rats. Immunohistochemistry assays indicated that carvedilol ameliorated intrahepatic angiogenesis. Transmission electron microscopy examination demonstrated that carvedilol improved sinusoidal remodeling. In the *in vitro* experiments, carvedilol suppressed transforming growth factor  $\beta$ 1 (TGF $\beta$ 1)-induced FN synthesis in HUVECs by inhibition of the TGF $\beta$ 1/Smads pathway.

**Conclusions:** Carvedilol ameliorated intrahepatic angiogenesis, sinusoidal remodeling and portal pressure in cirrhotic rats. Carvedilol improved sinusoidal remodeling by suppressing FN synthesis in endothelial cells. Carvedilol has potential utility for treating early-stage liver cirrhosis.

**MeSH Keywords:** **Endothelial Cells • Liver Cirrhosis • Portal Pressure**

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## Background

Liver cirrhosis has been recognized as a serious problem worldwide that notably affects patients' quality of life. Portal hypertension (PHT) is the most common and severe complication in patients with liver cirrhosis, resulting in severe clinical consequences, such as variceal bleeding [1,2]. Fortunately, great advances have been made in the physiopathology of liver cirrhosis and PHT. The prime determinant of PHT is increased intrahepatic vascular resistance (IHVR), which is caused by structural distortions and functional factors [3–7]. Structural factors including restructuring of the vasculature account for approximately 70% of the increase in IHVR in liver cirrhosis [3,8,9]. Vasculature restructuring includes intrahepatic angiogenesis and vascular remodeling, which are strongly associated with the progression of liver cirrhosis, IHVR and PHT [3,8,10]. Angiogenesis is defined as the growth of new vessels from existing ones, while the initial and most remarkable vascular remodeling occurs within hepatic sinusoids and has been referred to as sinusoidal remodeling [8]. The most prominent characteristic of sinusoidal remodeling is “capillarization” with the formation of organized basement membranes due to the deposition of matrix proteins in hepatic sinusoids [6]. Endothelial cells and cytokines play important roles in sinusoidal remodeling. In response to injuries, liver sinusoidal endothelial cells (LSECs) contribute to the formation of basement membranes by producing several types of matrix proteins including fibronectin (FN) [11–13]. Transforming growth factor  $\beta$ 1 (TGF $\beta$ 1) stimulates FN synthesis in LSECs [12,14]. Multiple molecules such as members of the Smad family, phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT), and extracellular signal-regulated kinase (ERK), are involved in TGF $\beta$ 1 signaling pathways [15,16].

Nonselective beta-blockers (NSBBs) are the first-line pharmacological treatment of PHT [17]. Carvedilol is a novel NSBB with additional anti- $\alpha_1$ -adrenergic activity and is more effective than traditional NSBBs (propranolol and nadolol) for the primary prophylaxis of variceal bleeding [18]. Recently, several studies in the cardiovascular field found that carvedilol could improve vascular structural remodeling [19–21]. Our previous study confirmed that carvedilol inhibited angiogenesis *in vitro* [22]. Therefore, we speculated that carvedilol could alter the intrahepatic vasculature in liver cirrhosis. We established a rat model of liver cirrhosis to investigate the effects of carvedilol on intrahepatic angiogenesis and sinusoidal remodeling. *In vitro*, we explored the mechanisms underlying the effect of carvedilol on sinusoidal remodeling.

## Material and Methods

### Animals

Forty male Wistar rats (180 g to 205 g) were obtained from the Central Animal Care Facility of Shandong University (Jinan, China). All rats were housed in the Laboratory Animal Center in Shandong Provincial Hospital affiliated with Shandong University. All rats were maintained in a temperature-controlled environment under a 12-hour light/dark cycle and had free access to food and water. All experimental procedures and protocols were approved by the Animal Medical Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University (Shandong China).

### Carbon tetrachloride (CCl<sub>4</sub>)-induced liver cirrhosis rat model

Liver cirrhosis was induced by intraperitoneal injection of CCl<sub>4</sub> (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) for 9 weeks as previously described [23]. Rats were randomly divided into 3 groups. Group 1 (n=10) was the control group; rats were injected (intraperitoneal) with olive oil (0.5 mL/kg) twice weekly. Group 2 (n=15) was the CCl<sub>4</sub>-treated group; rats were injected (intraperitoneal) with a mixture of CCl<sub>4</sub> and olive oil (CCl<sub>4</sub>: olive oil=1: 1, v/v; 0.1 mL/100 mg body weight) twice weekly. Group 3 (n=15) was the CCl<sub>4</sub>+carvedilol-treated group; rats were given CCl<sub>4</sub> in the same manner as rats in Group 2 and were concurrently co-treated with 10 mg/kg carvedilol (Qilu Pharmaceutical Company, Jinan, China) by gavage daily. In parallel, rats in Groups 1 and 2 were administered the same volume of saline by gavage daily.

### Cell culture

Human umbilical vein endothelial cells (HUVECs) were obtained from ATCC (Manassas, VA, USA) and cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), 100 mg/mL streptomycin, and 100 U/mL penicillin at 37°C in a humidified incubator with 5% CO<sub>2</sub>.

HUVECs were treated with or without the TGF $\beta$ 1 and different reagents. Carvedilol was purchased from Sigma-Aldrich (Saint Louis, MO, USA). Recombinant human TGF $\beta$ 1 was obtained from PeproTech (Rocky Hill, NJ, USA). The inhibitors PD98059 and SIS3 were both purchased from MedChem Express (Monmouth Junction, NJ, USA).

### Measurement of portal pressure

After anesthesia induction by intraperitoneal injection of pentobarbital (30 mg/kg), the rats received a laparotomy to expose the portal vein. A PE-50 catheter was inserted into the

portal vein to measure the portal pressure. The catheter was connected to a pressure transducer. The portal pressure was recorded by a multichannel recorder (BeneView T8, Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) [24]. The pressure measurement lasted for 1 minute. The average value was used as the final portal pressure value. After data acquisition, the rats were sacrificed.

### Immunohistochemistry

We used a Polink-2 plus Polymer-Horseradish Peroxidase (HRP) Anti-Rabbit IgG Detection system (Zhongshan Golden Bridge, Beijing, China) for the immunohistochemistry analysis. The procedure was performed according to the manufacturer's instructions. Paraffin-embedded liver slides were deparaffinized and rehydrated. Heat-mediated antigen retrieval was performed with Tris/EDTA buffer, pH 9.0. After blocking the endogenous peroxidase activity, the slides were incubated with CD31 primary antibody (Abcam, Cambridge, UK; ab182981, 1: 500) at 4°C overnight. Following incubation with a specific secondary antibody at 37°C for 30 minutes, the slides were developed by incubation with diaminobenzidine (DAB). Then, the slides were counterstained with hematoxylin and visualized under a light microscope (OLYMPUS BX63F, Tokyo, Japan). The negative control sections were incubated with phosphate-buffered saline (PBS) instead of CD31 primary antibody. The expression of CD31 was quantified by ImageJ software (National Institutes of Health, Bethesda, MD, USA).

### Transmission electron microscopy

The medial lobe of the liver was cut into small blocks and fixed in 0.1 M phosphate buffer (pH 7.4) containing 2.0% glutaraldehyde for 4 hours. Then, the blocks were fixed in osmium for 4 hours. After dehydration in a graded series of alcohols, the specimens were embedded in Spurr's resin and sectioned. Five samples from each group were examined with a transmission electron microscope (JEOL-1200EX, Japan) at 15 000× magnification.

### RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the treated HUVECs using the TRIzol reagent (Takara Biotechnology Co., Ltd., Dalian, China). A PrimeScript™ RT reagent Kit (Takara Biotechnology) was used for reverse transcription according to the manufacturer's instructions. A SYBR Premix Ex Tap Kit (Takara Biotechnology) was used for amplification. The qRT-PCR reaction was conducted with a LightCycler 480 Real-Time PCR system (Roche Diagnostics, USA). The expression level of target genes was calculated using the  $2^{-\Delta\Delta CT}$  method and normalized to that of glyceraldehyde phosphate dehydrogenase (GAPDH). The primer sequences used

are as follows: FN forward, 5'-GATAAATCAACAGTGGGAGC-3' and reverse, 5'-CCCAGATCATGGAGTCTTTA-3'; GAPDH forward, 5'-GCACCGTCAAGGCTGAGAAC-3' and reverse, 5'-TGGTGAAGACGCCAGTGGA-3'.

### Protein extraction and western blot analysis

HUVECs were seeded into 6-well culture plates. After treatment, the M-PER™ Mammalian Protein Extraction reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used for protein extraction. Protein concentrations were measured using a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology, Haimen, China) according to the manufacturer's protocol. Equal quantities of protein (50 µg) were separated by 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to microporous polyvinylidene fluoride (PVDF) membranes (EMD Millipore, MA, USA). After being blocked in 5% skimmed milk at room temperature for 1 hour, the membranes were incubated with primary antibodies at 4°C overnight and then incubated with HRP-conjugated goat anti-rabbit or anti-mouse IgG secondary antibodies (Zhongshan Golden Bridge, Beijing, China) for 1 hour at room temperature. The bands were detected via enhanced chemiluminescence using an Amersham Imager 600 (GE Healthcare, USA). The expression of target proteins was normalized to that of GAPDH. The primary antibodies used include the following: anti-FN (ab2413, 1: 1000), anti-total Smad2 (ab40855, 1: 2000), and anti-phospho-Smad3 (ab52903, 1: 2000), which were purchased from Abcam (Cambridge, UK); anti-total AKT (#4691, 1: 1000), anti-phospho-AKT (#4060, 1: 1000), anti-total-p44/42 MAPK (t-ERK1/2, #9102, 1: 1000), anti-phospho-p44/42 MAPK (p-ERK1/2, #9101, 1: 1000), anti-total Smad3 (#9523, 1: 1000), anti-phospho-Smad2 (#3108, 1: 1000), and anti-GAPDH (#5174, 1: 1000), which were purchased from Cell Signaling Technology (Bedford, USA).

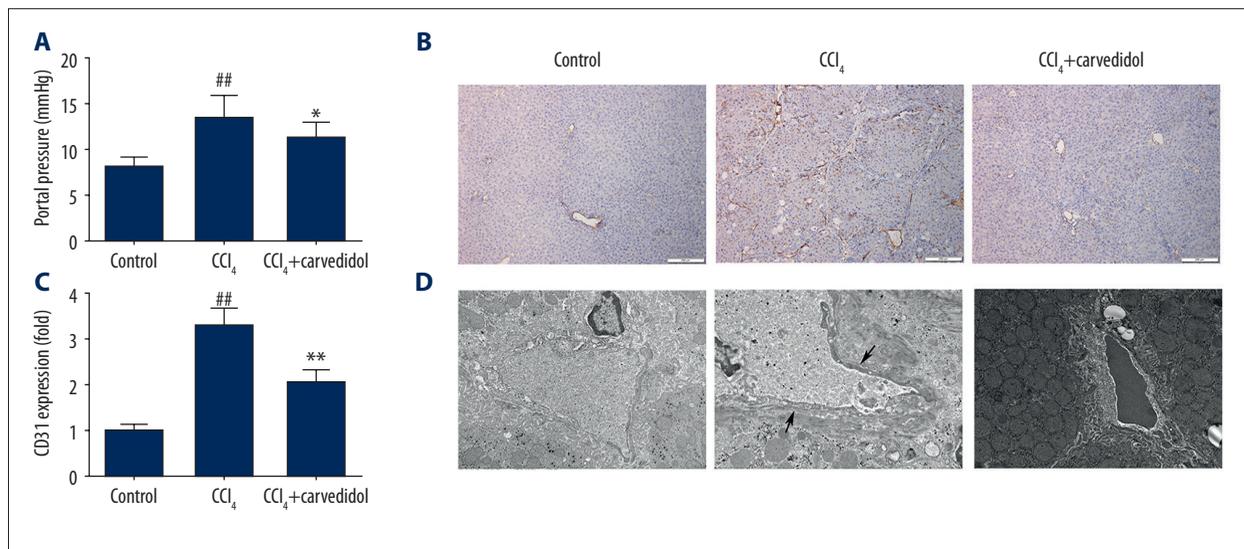
### Statistical analysis

All data are expressed as means ± standard deviation (SD). SPSS Statistics 20.0 (SPSS Inc., IBM, Armonk, NY, USA) was used for the statistical analyses. Multiple comparisons were analyzed by one-way ANOVA followed by Dunnett's test. Statistical significance was accepted at  $P < 0.05$ .

## Results

### Carvedilol reduced portal pressure in cirrhotic rats

As shown in Figure 1A, the portal pressure of rats in the CCl<sub>4</sub>-treated cirrhotic group was markedly higher than that of rats in the control group ( $P < 0.01$ ), while concurrent treatment with carvedilol reduced the elevated portal pressure in cirrhotic



**Figure 1.** Effects of carvedilol on portal pressure, intrahepatic angiogenesis, and sinusoidal remodeling in cirrhotic rats. (A) Measurement of portal pressure in rats. (B, C) CD31 immunohistochemistry in liver sections (magnification 100×). (D) Images from transmission electron microscopy (magnification 15 000×). Arrows indicate the formation of organized basement membranes on the basal side of LSECs. A total of 6 to 8 rats were included in each group (<sup>##</sup>  $P < 0.01$  versus the control group, <sup>\*</sup>  $P < 0.05$  versus the CCl<sub>4</sub> group, <sup>\*\*</sup>  $P < 0.01$  versus the CCl<sub>4</sub> group).

rats ( $P < 0.05$ ). The results indicated that carvedilol effectively reduced the portal pressure in cirrhotic rats.

#### Carvedilol inhibited intrahepatic angiogenesis in cirrhotic rats

Intrahepatic angiogenesis was assessed by examining the expression of CD31, a marker of vascular endothelial cells. Immunohistochemical analysis showed normal angioarchitecture in the livers of control rats, in which CD31 was expressed in the endothelium of the vessels and along the hepatic sinusoids. The livers of CCl<sub>4</sub>-treated cirrhotic rats exhibited abnormal vascular structures with an increasing number of vessels of varying diameter, while the disorganized vascular network in the livers of CCl<sub>4</sub>+carvedilol-treated rats was significantly improved (Figure 1B). Semiquantitative analysis of CD31 expression showed that the livers of CCl<sub>4</sub>-treated cirrhotic rats exhibited a higher CD31 expression level than those of control rats ( $P < 0.01$ ), and concurrent treatment with carvedilol ameliorated the increased CD31 expression induced by CCl<sub>4</sub> ( $P < 0.01$ ) (Figure 1B, 1C). The results supported that carvedilol inhibited abnormal intrahepatic angiogenesis in cirrhotic livers.

#### Carvedilol alleviated sinusoidal remodeling in cirrhotic rats

The formation of basement membranes on the basal side of LSECs in the space of Disse was examined by transmission electron microscopy. The livers of control rats showed no obvious deposition of matrix proteins in the perisinusoidal space of the hepatic parenchyma, and LSECs lacked basement membranes.

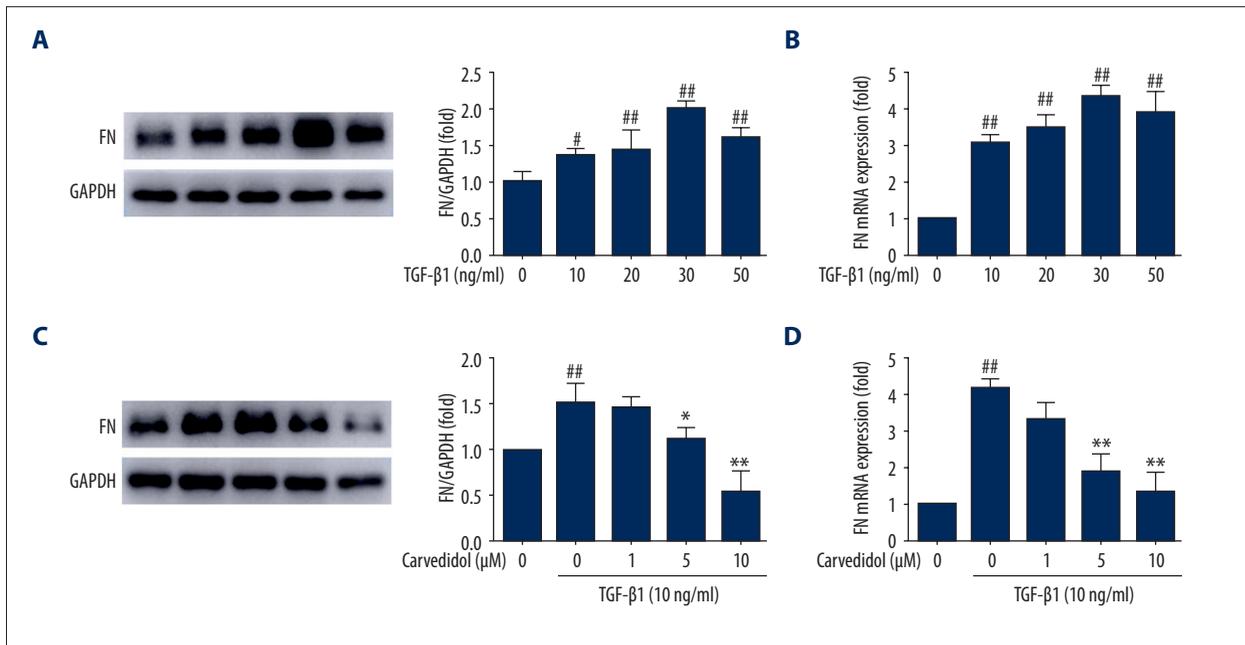
In the liver tissues from CCl<sub>4</sub>-treated cirrhotic rats, the formation of complete basement membranes was observed on the abluminal surface of LSECs due to the excessive and abnormal deposition of matrix proteins in the space of Disse (Figure 1D). Administration of carvedilol decreased the abnormal deposition of matrix proteins induced by CCl<sub>4</sub> in the space of Disse, and the basement membranes on the basal side of LSECs in the livers of CCl<sub>4</sub>+carvedilol-treated rats were discontinuous and unapparent (Figure 1D). The results demonstrated that carvedilol alleviated sinusoidal remodeling in cirrhotic rats.

#### Carvedilol inhibited TGFβ1-induced FN synthesis in HUVECs

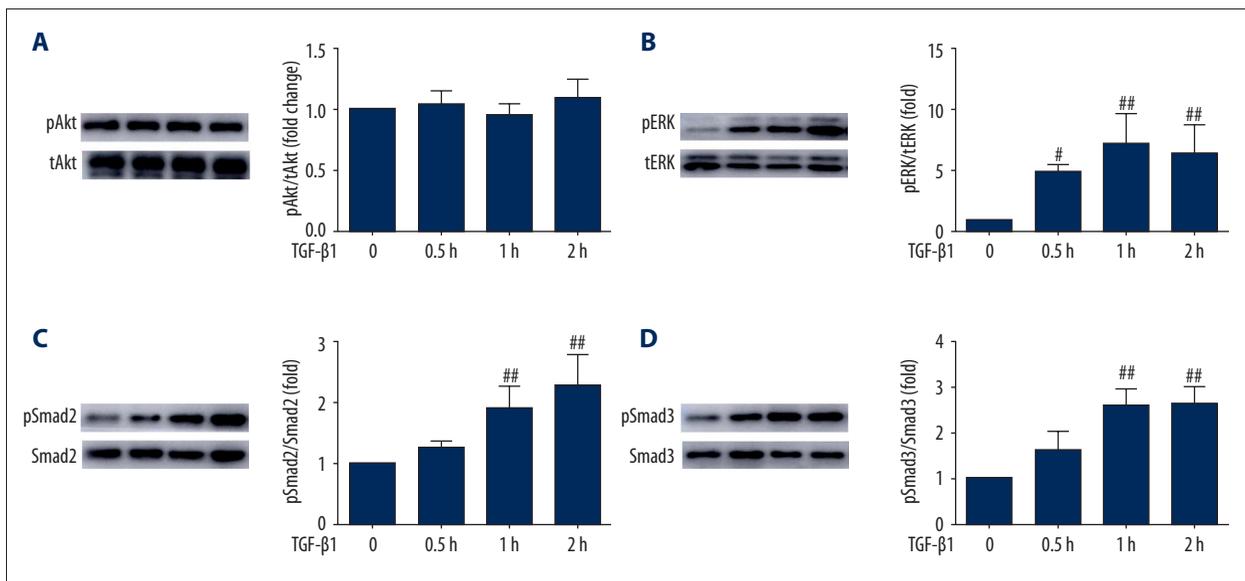
After incubation with various concentrations of TGFβ1 for 24 hours, the protein and mRNA expression levels of FN in the TGFβ1-treated group were significantly upregulated compared to those of the control group ( $P < 0.05$  and  $P < 0.01$ , respectively) (Figure 2A, 2B). The cells were pretreated with carvedilol for 2 hours before incubation with 10 ng/mL TGFβ1 for 24 hours. As shown in Figure 2C and 2D, pretreatment with 5 and 10 μM carvedilol reduced the TGFβ1-induced upregulation of FN protein and mRNA ( $P < 0.05$  and  $P < 0.01$ , respectively).

#### Carvedilol inhibited the TGFβ1/Smads and ERK pathways in HUVECs

After stimulation with TGFβ1 for different time intervals, the expression levels of phosphorylated ERK, Smad2, and Smad3 were significantly upregulated compared to those of the control group ( $P < 0.05$  and  $P < 0.01$ , respectively) (Figure 3B–3D),



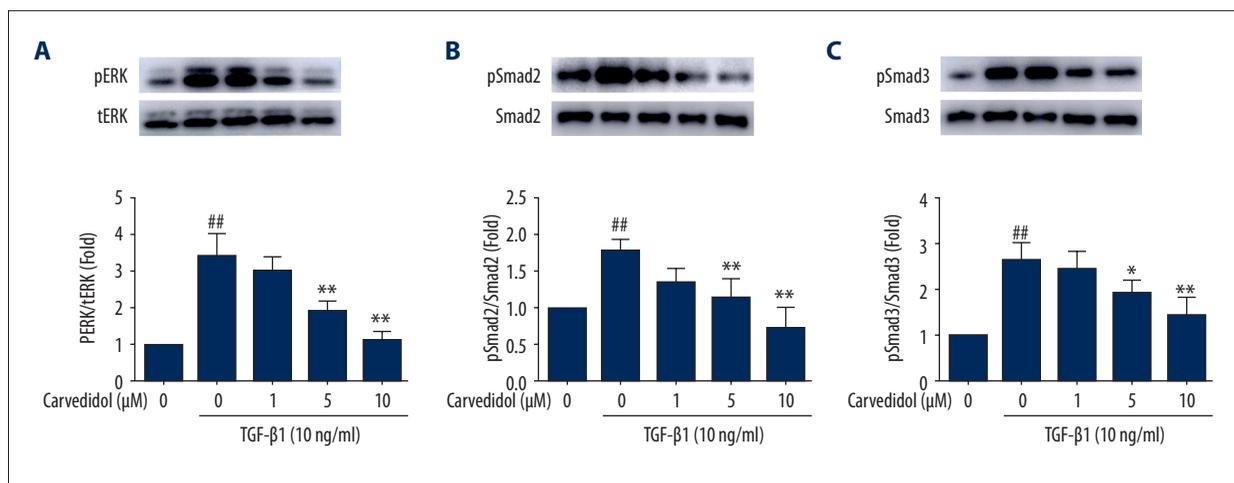
**Figure 2.** Carvedilol suppressed the upregulation of FN protein and mRNA expression induced by TGFβ1 in HUVECs. (A) FN protein expression induced by TGFβ1 in HUVECs. (B) FN RNA expression induced by TGFβ1. (C) FN protein expression in HUVECs pretreated with carvedilol. (D) FN mRNA expression in HUVECs pretreated with carvedilol. Each experiment was repeated 3 times (#  $P < 0.05$  versus control, ##  $P < 0.01$  versus control, \*  $P < 0.05$  versus TGFβ1, \*\*  $P < 0.01$  versus TGFβ1).



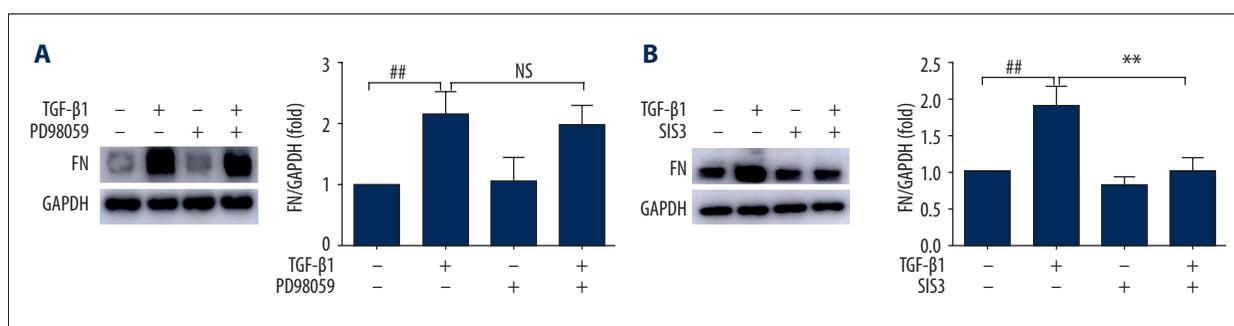
**Figure 3.** The protein expression of phosphorylated AKT, ERK, Smad2 and Smad3 in HUVECs induced by TGFβ1. (A) Phosphorylated AKT protein expression. (B) Phosphorylated ERK protein expression. (C) Phosphorylated Smad2 protein expression. (D) Phosphorylated Smad3 protein expression. Each experiment was repeated 3 times (#  $P < 0.05$  versus control, ##  $P < 0.01$  versus control).

while no change was found in the expression of phosphorylated AKT ( $P > 0.05$ ) (Figure 3A). The cells were pretreated with carvedilol for 2 hours before TGFβ1 stimulation for 0.5 hours. As shown in Figure 4, pretreatment with carvedilol at 5 μM and 10 μM decreased TGFβ1-induced phosphorylation ERK,

Smad2 and Smad3 ( $P < 0.05$  and  $P < 0.01$ , respectively). The results suggested that the Smads and ERK pathways were involved in TGFβ1-induced FN synthesis in HUVECs.



**Figure 4.** Carvedilol suppressed the protein expression of phosphorylated AKT, ERK Smad2, and Smad3 induced by TGFβ1 in HUVECs. (A) Phosphorylated ERK protein expression. (B) Phosphorylated Smad2 protein expression. (C) Phosphorylated Smad3 protein expression. Each experiment was repeated 3 times (##  $P < 0.01$  versus control, \*  $P < 0.05$  versus TGFβ1, \*\*  $P < 0.01$  versus TGFβ1).



**Figure 5.** Effects of PD98059 and SIS3 on TGFβ1-induced FN synthesis in HUVECs. (A) FN expression in HUVECs pretreated with PD98059. (B) FN expression in HUVECs pretreated with SIS3. Each experiment was repeated 3 times (##  $P < 0.01$  versus control, \*\*  $P < 0.01$  versus TGF β1, NS not statistically significant).

### Carvedilol suppressed TGFβ1-induced FN synthesis in HUVECs through inhibition of the TGFβ1/Smads pathway

The effects of PD98059 (a potent inhibitor of ERK, 10 μM) and SIS3 (a selective inhibitor of Smad3, 10 μM) on TGFβ1-induced FN synthesis were investigated. After incubation with TGFβ1 for 24 hours, the expression of FN was significantly increased compared to that of the control group ( $P < 0.01$ ), while co-treatment with PD98059 did not alter FN expression ( $P > 0.05$ ) (Figure 5A). As demonstrated in Figure 5B, TGFβ1 upregulated the expression of FN compared to the control group ( $P < 0.01$ ), while co-culture with SIS3 almost completely prevented this effect ( $P < 0.01$ ). The results suggested that carvedilol suppresses TGFβ1-induced FN synthesis by targeting the TGFβ1/Smads pathway.

### Discussion

The present study shows, for the first time, that carvedilol ameliorates intrahepatic angiogenesis, sinusoidal remodeling, and

portal pressure in cirrhotic rats. In the *in vitro* study, carvedilol improved sinusoidal remodeling by inhibiting FN synthesis of endothelial cells by targeting the TGFβ1/Smads pathway.

In recent years, studies have revealed that PHT is a consequence of complex processes including intrahepatic angiogenesis and sinusoidal remodeling [6–8,25]. Many disordered, newly formed vessels bypass hepatic sinusoids in response to the structural distortion of the liver, which increases the IHVR to portal blood flow [25]. Healthy livers have a low density of matrix proteins in the space of Disse, and LSECs lack a basement membrane in the endothelium [13]. In the early stage of liver fibrosis, the excessive matrix proteins are deposited on the basal side of LSECs and form continuous basement membranes. This pathogenetic sinusoidal remodeling disables the transport and exchange of nutrients from the lumen of the hepatic sinusoid to the space of Disse and weakens the adaption of LSECs to the increased resistance to blood flow [13]. Multiple tyrosine kinase receptor inhibitors, such as sorafenib and sunitinib, reduce portal pressure and markedly reduce intrahepatic

inflammation, fibrosis, and angiogenesis in cirrhotic and portal hypertensive rats [26,27]. Atorvastatin inhibits angiogenesis and decreases portal pressure in cirrhotic (bile duct ligation; CCl<sub>4</sub> intoxication) rats [28]. Improvement of hepatic sinusoidal capillarization can decrease the portal vein pressure in thioacetamide-induced cirrhotic rats [8,29]. These studies highlighted that pharmacologic interventions targeting angiogenesis and sinusoidal remodeling could reduce portal pressure in portal hypertensive and cirrhotic animal models. Our previous study confirmed that carvedilol exhibits an antiangiogenic effect *in vitro* [22]. The present study demonstrated that carvedilol inhibits intrahepatic angiogenesis and sinusoidal remodeling in cirrhotic rats, accompanied by a decrease in portal pressure. We speculate that carvedilol reduces portal pressure partly through improvement of angiogenesis and sinusoidal remodeling.

FN is an important component of matrix proteins because it forms a continuous layer in the space of Disse [30]. As the most potent profibrogenic cytokine in the liver, TGFβ1 stimulates FN synthesis in LSECs during the early stage of liver injury [12,31–33]. TGFβ1 can activate the Smad-dependent pathway and induce activation of Smad-independent pathways such as ERK, c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (p38 MAPK), and PI3K/AKT [15,34–36]. The Smad-dependent pathway is the most important; after stimulation by TGFβ1, Smad2 and Smad3 are phosphorylated, and the 2 phosphorylated Smads, along with Smad4, form a heterotrimeric complex that translocates into the nucleus and regulates the expression of target genes [15,36]. Recently, several studies have examined the molecular mechanisms of pharmacologic interventions on TGFβ1-induced FN synthesis.

## References:

- Sanyal AJ, Bosch J, Blei A, Arroyo V: Portal hypertension and its complications. *Gastroenterology*, 2008; 134: 1715–28
- Bosch J, Garcia-Pagan JC: Complications of cirrhosis. I. Portal hypertension. *J Hepatol*, 2000; 32: 141–56
- Bosch J, Groszmann RJ, Shah VH: Evolution in the understanding of the pathophysiological basis of portal hypertension: How changes in paradigm are leading to successful new treatments. *J Hepatol*, 2015; 62: S121–30
- Fernandez M: Molecular pathophysiology of portal hypertension. *Hepatology*, 2015; 61: 1406–15
- Garcia-Pagan JC, Gracia-Sancho J, Bosch J: Functional aspects on the pathophysiology of portal hypertension in cirrhosis. *J Hepatol*, 2012; 57: 458–61
- Iwakiri Y, Shah V, Rockey DC: Vascular pathobiology in chronic liver disease and cirrhosis – current status and future directions. *J Hepatol*, 2014; 61: 912–24
- Garbuzenko DV, Arefyev NO, Belov DV: Mechanisms of adaptation of the hepatic vasculature to the deteriorating conditions of blood circulation in liver cirrhosis. *World J Hepatol*, 2016; 8: 665–72
- Thabut D, Shah V: Intrahepatic angiogenesis and sinusoidal remodeling in chronic liver disease: New targets for the treatment of portal hypertension? *J Hepatol*, 2010; 53: 976–80
- Garcia-Tsao G, Abraldes JG, Berzigotti A, Bosch J: Portal hypertensive bleeding in cirrhosis: Risk stratification, diagnosis, and management: 2016 practice guidance by the American Association for the study of liver diseases. *Hepatology*, 2017; 65: 310–35
- Fernandez M, Semela D, Bruix J et al: Angiogenesis in liver disease. *J Hepatol*, 2009; 50: 604–20
- Jarnagin WR, Rockey DC, Koteliensky VE et al: Expression of variant fibronectins in wound healing: Cellular source and biological activity of the E11A segment in rat hepatic fibrogenesis. *J Cell Biol*, 1994; 127: 2037–48
- Neubauer K, Kruger M, Quondamatteo F et al: Transforming growth factor-beta1 stimulates the synthesis of basement membrane proteins laminin, collagen type IV and entactin in rat liver sinusoidal endothelial cells. *J Hepatol*, 1999; 31: 692–702
- Natarajan V, Harris EN, Kidambi S: SECs (sinusoidal endothelial cells), liver microenvironment, and fibrosis. *BioMed Research International*, 2017; 2017: 4097205
- Hahn E, Wick G, Pencev D, Timpl R: Distribution of basement membrane proteins in normal and fibrotic human liver: Collagen type IV, laminin, and fibronectin. *Gut*, 1980; 21: 63–71
- Derynck R, Zhang YE: Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature*, 2003; 425: 577–84
- Kajdaniuk D, Marek B, Borgiel-Marek H, Kos-Kudla B: Transforming growth factor beta1 (TGFbeta1) in physiology and pathology. *Endokrynol Pol*, 2013; 64: 384–96

## Conclusions

The present study is the first to demonstrate that carvedilol ameliorates intrahepatic angiogenesis, sinusoidal remodeling, and portal pressure in cirrhotic rats. The results indicated that the improvements in intrahepatic vascular structure are in part involved in the mechanisms of carvedilol with regard to PHT. This study provided experimental evidence that reagents targeting angiogenesis and sinusoidal remodeling could reduce portal pressure. Our research strongly supports the idea that the application of carvedilol for liver cirrhosis could be extended beyond the pharmacological treatment of PHT. We propose that carvedilol may be used in the treatment of early-stage liver cirrhosis.

## Conflicts of interest

None.

17. Tripathi D, Hayes PC: Beta-blockers in portal hypertension: New developments and controversies. *Liver Int*, 2014; 34: 655–67
18. de Franchis R: Expanding consensus in portal hypertension: Report of the Baveno VI Consensus Workshop: Stratifying risk and individualizing care for portal hypertension. *J Hepatol*, 2015; 63: 743–52
19. Kishihara J, Niwano S, Niwano H et al: Effect of carvedilol on atrial remodeling in canine model of atrial fibrillation. *Cardiovasc Diagn Ther*, 2014; 4: 28–35
20. Wang K, Lu D, Zhang B et al: Renal denervation attenuates multi-organ fibrosis and improves vascular remodeling in rats with transverse aortic constriction induced cardiomyopathy. *Cell Physiol Biochem*, 2016; 40: 465–76
21. Cao G, Llambi HG, Ottaviano G et al: Carvedilol protects the peritubular capillaries and kidney structure in spontaneously hypertensive rats. *Int J Cardiol*, 2015; 181: 415–16
22. Ding Q, Tian XG, Li Y et al: Carvedilol may attenuate liver cirrhosis by inhibiting angiogenesis through the VEGF-Src-ERK signaling pathway. *World J Gastroenterol*, 2015; 21: 9566–76
23. Starkel P, Leclercq IA: Animal models for the study of hepatic fibrosis. *Best Pract Res Clin Gastroenterol*, 2011; 25: 319–33
24. Chen Y, Wang W, Wang H et al: Rapamycin attenuates splenomegaly in both intrahepatic and prehepatic portal hypertensive rats by blocking mTOR signaling pathway. *PLoS One*, 2016; 11: e0141159
25. Garbuzenko DV, Arefyev NO, Kazachkov EL: Antiangiogenic therapy for portal hypertension in liver cirrhosis: Current progress and perspectives. *World J Gastroenterol*, 2018; 24: 3738–48
26. Mejias M, Garcia-Pras E, Tiani C et al: Beneficial effects of sorafenib on splanchnic, intrahepatic, and portocollateral circulations in portal hypertensive and cirrhotic rats. *Hepatology*, 2009; 49: 1245–56
27. Tugues S, Fernandez-Varo G, Munoz-Luque J et al: Antiangiogenic treatment with sunitinib ameliorates inflammatory infiltrate, fibrosis, and portal pressure in cirrhotic rats. *Hepatology*, 2007; 46: 1919–26
28. Uschner FE, Ranabhat G, Choi SS et al: Statins activate the canonical hedgehog-signaling and aggravate non-cirrhotic portal hypertension, but inhibit the non-canonical hedgehog signaling and cirrhotic portal hypertension. *Sci Rep*, 2015; 5: 14573
29. Xu H, Shi BM, Lu XF et al: Vascular endothelial growth factor attenuates hepatic sinusoidal capillarization in thioacetamide-induced cirrhotic rats. *World J Gastroenterol*, 2008; 14: 2349–57
30. Clement B, Grimaud JA, Campion JP et al: Cell types involved in collagen and fibronectin production in normal and fibrotic human liver. *Hepatology*, 1986; 6: 225–34
31. Smedsrod B, De Bleser PJ, Braet F et al: Cell biology of liver endothelial and Kupffer cells. *Gut*, 1994; 35: 1509–16
32. Rieder H, Ramadori G, Dienes HP et al: Sinusoidal endothelial cells from guinea pig liver synthesize and secrete cellular fibronectin *in vitro*. *Hepatology*, 1987; 7: 856–64
33. Liu XY, Liu RX, Hou F et al: Fibronectin expression is critical for liver fibrogenesis *in vivo* and *in vitro*. *Mol Med Rep*, 2016; 14: 3669–75
34. Xu F, Liu C, Zhou D, Zhang L: TGF-beta/SMAD pathway and its regulation in hepatic fibrosis. *J Histochem Cytochem*, 2016; 64: 157–67
35. Bakin AV, Tomlinson AK, Bhowmick NA et al: Phosphatidylinositol 3-kinase function is required for transforming growth factor beta-mediated epithelial to mesenchymal transition and cell migration. *J Biol Chem*, 2000; 275: 36803–10
36. Inagaki Y, Okazaki I: Emerging insights into transforming growth factor beta Smad signal in hepatic fibrogenesis. *Gut*, 2007; 56: 284–92
37. Lin SH, Chen IJ, Chuang CT et al: KMUP-1 attenuates high glucose and transforming growth factor-beta1-induced pro-fibrotic proteins in mesangial cells. *Mol Med Rep*, 2017; 15: 4199–206
38. Yadav UC, Naura AS, Aguilera-Aguirre L et al: Aldose reductase inhibition prevents allergic airway remodeling through PI3K/AKT/GSK3beta pathway in mice. *PLoS One*, 2013; 8: e57442