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ORIGINAL ARTICLE

Lycopene treatment improves intrahepatic fibrosis and attenuates pathological angiogenesis in biliary cirrhotic rats

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Abstract

Background: Liver cirrhosis is characterized by liver fibrosis and pathological angiogenesis, which results in hyperdynamic circulation, portal-systemic collateral vascular formation, and abnormal angiogenesis. Lycopene is a nutrient mostly found in tomatoes. The beneficial effects of lycopene include anti-inflammation, anti-oxidation, anti-fibrosis, and anti-angiogenesis; however, the association between liver cirrhosis and pathological angiogenesis has yet to be studied. This study aimed to investigate the effects of lycopene on biliary cirrhotic rats.

Methods: The efficacy of lycopene treatment in common bile duct ligation (BDL)-induced biliary cirrhotic rats was evaluated. Sham-operated rats served as surgical controls. Lycopene (20 mg/kg/day, oral gavage) or vehicle was administered to BDL or sham-operated rats for 4 weeks, after which the hemodynamics, liver biochemistry, portal-systemic shunting, liver and mesenteric angiogenesis, and hepatic angiogenesis-related protein expressions were examined.

Results: Lycopene alleviated hyperdynamic circulation as evidenced by decreased cardiac index and increased peripheral vascular resistance ($p < 0.05$), but it did not affect portal pressure or liver biochemistry in the BDL rats ($p > 0.05$). Lycopene significantly diminished the shunting degree of portal-systemic collaterals ($p = 0.04$) and mesenteric vascular density ($p = 0.01$), and also ameliorated intrahepatic angiogenesis and liver fibrosis. In addition, lycopene upregulated endothelial nitric oxide synthase, protein kinase B (Akt) and phosphatidylinositol 3-kinases (PI3K), and downregulated vascular endothelial growth factor receptor 2 (VEGFR-2) protein expressions ($p < 0.05$) in the livers of the BDL rats.

Conclusion: Lycopene ameliorated liver fibrosis, hyperdynamic circulation, and pathological angiogenesis in biliary cirrhotic rats, possibly through the modulation of intrahepatic Akt/PI3K/eNOS and VEGFR-2 pathways.

Keywords: Angiogenesis; Hyperdynamic circulation; Liver cirrhosis; Lycopene; Portal hypertension

1. INTRODUCTION

Various kinds of acute and chronic liver injuries elicit tissue responses aimed at replacing the damaged cells. However, the process is usually suboptimal and fibrogenesis ensues, followed by an increase in intrahepatic resistance. This increase in intrahepatic resistance can be attributed to structural derangements caused by collagen fiber deposition and functional derangements caused by intrahepatic vasoconstriction.[1] Apart from the increase in intrahepatic resistance, increases in the release of splanchnic and peripheral vascular vasodilatory substances significantly elevate portal inflow and portal pressure (PP) in liver cirrhosis.[2] This then triggers the formation of portal-systemic collaterals and abnormal growth of mesenteric vasculature.[3] Liver cirrhosis causes circulatory abnormalities, the so-called hyperdynamic circulation, which is characterized by an increase in cardiac output and decreases in blood pressure and systemic vascular resistance.[4] Emerging data show that hyperdynamic circulation is related to systemic and intrahepatic angiogenesis in liver cirrhosis.[5] The pathological angiogenesis and vascular remodeling contribute to cirrhotic complications including portal hypertension and esophageal variceal bleeding. Angiogenesis also plays a pivotal role in portal-systemic collateral circulation, mesenteric vasculature, and hyperdynamic circulation.

Lycopene is a bioactive carotenoid found in a variety of fruits and vegetables such as tomatoes, watermelons, and grapefruits,[6] of which tomatoes are the major dietary source. Lycopene has

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been linked with the prevention of cardiovascular diseases due to its pleiotropic effects.[7] Lycopene has been demonstrated to inhibit hepatic stellate cell activation, which is the main action of a damaged liver towards fibrogenesis.[8] Anti-angiogenesis is another remarkable feature of lycopene. Lycopene has been shown to significantly decrease endothelial cell proliferation, migration, and tube formation, suggesting that it may inhibit angiogenesis.[9] Moreover, physiological concentrations of lycopene have been shown to inhibit *in vitro* angiogenesis in human umbilical vein endothelial cells as well as in rat aortic rings.[10] Lycopene has also been shown to decrease hypoxia-induced factor, vascular endothelial growth factor, matrix metalloproteinase, and alpha fetoprotein expression in a murine model of hepatocellular carcinoma.[11]

Although lycopene has been shown to exert anti-angiogenesis and potentially anti-fibrogenesis effects in various experimental models, its influence on pathological angiogenesis in cirrhosis remains unexplored. We hypothesized that lycopene could ameliorate hemodynamic derangements, liver fibrosis and diminish abnormal liver, and mesentery angiogenesis in liver cirrhosis. Therefore, the aim of this study was to investigate whether lycopene treatment can influence liver fibrosis, portal hypertension-related hyperdynamic circulation and abnormal angiogenesis in cirrhotic rats.

2. METHODS

2.1. Animal Model

Male Sprague-Dawley rats weighing 280–300 g were used in this study. The rats were caged at 24°C, with a 12-h light-dark cycle and free access to food and water until the time of experiments. A common bile duct ligation (BDL)-induced biliary cirrhosis animal model was used in this study. Survival surgery and hemodynamic studies were performed under anesthesia with Zoletil-100 injection intramuscularly (50 mg/kg). This study was authorized by the Animal Committee of our hospital (IACUC 2015-221, 2019-061). All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals, 8th edition, 2011" published by the National Research Council, the United States.

2.2. Common BDL and Sham Operations

Secondary biliary cirrhosis was induced using common BDL as previously described.[12] A high yield of secondary biliary cirrhosis was noted 4 weeks after the ligation. To avoid coagulation defects, the BDL rats received weekly vitamin K injections (50 μg/kg intramuscularly). The sham-operated rats received a sham operation without ligation of the common bile duct.

2.3. Study Protocol

Series 1: Lycopene was diluted with olive oil or vehicle (olive oil, control) and administered to the sham-operated or BDL rats at a dose of 20 mg/kg once a day via oral gavage from the first day of BDL or sham operation to the 28th day. After 4 weeks of treatment, body weight, blood biochemistry, and hemodynamic data were measured.

Series 2: After 4 weeks of lycopene or vehicle treatment, comprehensive hemodynamic data were recorded in the BDL and sham-operated rats. Hepatic histopathology and protein expression analyses, and liver fibrosis and immunohistochemistry studies for intrahepatic angiogenesis were also performed.

Series 3: After 4 weeks of lycopene or vehicle treatment, the shunting degree of portal-systemic collaterals and vascular density of mesenteric vasculature were determined in the BDL rats.

2.4. Measurement of Systemic and Portal Hemodynamics

The right femoral artery and superior mesentery vein were cannulated with PE-50 catheters that were connected to a Spectramed DTX transducer (Spectramed Inc., Oxnard, CA, USA). Continuous recordings of mean arterial pressure (MAP), heart rate (HR), and PP were performed on a multichannel recorder (model RS 3400, Gould Inc., Cupertino, CA, USA). Cardiac output (ml/min) was measured using a thermodilution method as previously described.[13] Cardiac index (CI, 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 as MAP divided by CI. Superior mesentery arterial resistance (mmHg/ml/min/100 g BW) was calculated as (MAP-PP)/superior mesentery artery flow per 100 g BW.

2.5. Portal Venous Flow and Superior Mesenteric Artery Flow Measurement

The measurements of portal venous flow (ml/min/100 g BW) and superior mesentery artery flow (ml/min/100 g BW) were performed using a non-constrictive perivascular ultrasonic transit-time flow probe (lRB, 1-mm diameter; Transonic Systems, Ithaca, NY, USA).

2.6. Hepatic Hematoxylin and Eosin Staining and Determination of Fibrosis

Liver tissue specimens were fixed in 10% formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin (H&E). The liver paraffin sections were then stained using a Sirius Red staining kit (Polysciences Inc., Warrington, PA, USA), and the severity of liver fibrosis was determined.

2.7. Immunohistochemical Study of Intrahepatic Angiogenesis

To determine intrahepatic angiogenesis, immunohistochemical staining with anti-CD31 antibodies (1:200, Serotec and Pharmingen, San Diego, CA, USA) was performed,[14] using the primary antibodies against CD-31 followed by biotinylated anti-mouse IgG (H+L) (Vector Laboratories, Burlingame, CA, USA) as the second antibody.

2.8. Western Blot Analysis

Liver tissues were frozen in liquid nitrogen and stored at -80°C until required for Western blot analysis. Blots were incubated with the primary antibody [endothelial nitric oxide synthase (eNOS) (Cell Signaling 32027S; 1:1000), inducible nitric oxide synthase (iNOS) (Genetex Gtx130246; 1:1000), phosphatidylinositol 3-kinases (PI3K) (Abcam ab61224; 1:1000), protein kinase B (Akt) (Cell Signaling 4691L; 1:3000), extracellular signal-regulated kinase (ERK) (Cell Signaling 9102S; 1:3000), vascular endothelial growth factor receptor-2 (VEGFR-2) (Millipore Abs82; 1:3000), and beta-actin (Genetex Gtx629630; 1:5000)], and then the blots were incubated for 90 min with secondary antibody (horseradish peroxidase-conjugated goat anti-mouse IgG antibody; Sigma Chemical Co., St. Louis, MO, USA). The specific proteins were detected by enhanced chemiluminescence (Immobilon Western Chemiluminescent HRP Substrate, Merk Millipore Co., Billerica, MA, USA) and scanned with a computer-assisted video densitometer and digitalized system (BioSpectrum® 600 Imaging System, Ultra-Violet Products Ltd., Upland, CA, USA). The signal intensity (integral volume) of the appropriate band was then analyzed.

2.9. Portal-systemic Shunting Analysis

Portal-systemic shunting was determined using the color microsphere method.[15] The degree of portal-systemic shunting was calculated as the number of microspheres in the lung divided by the sum of microspheres in the liver and lung.

2.10. Immunofluorescent Study for Mesenteric Vascular Density

Mesenteric vascular angiogenesis was quantified using CD31-labelled microvascular networks.[16,17] Primary antibody mouse anti-rat CD31-biotin (1:200; AbD Serotec, Oxford, UK) and secondary antibody (CY2-conjugated streptavidin, 1:1000; Jackson ImmunoResearch, West Grove, PA, USA) were applied.

2.11. Drugs

Lycopene was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solutions were freshly prepared on the days of the experiments.

2.12. Statistical Analysis

All results are expressed as mean ± SD. Statistical analyses were performed using an unpaired Student's t-test for the comparisons of rats without (sham) or with (BDL) liver cirrhosis and hepatic protein expressions, portal-systemic shunting, and mesenteric vascular density in BDL rats with vehicle or lycopene treatment. The one-way ANOVA with the least significant difference test was performed for statistical analyses of hemodynamic and biochemistry data in sham-operated and BDL rats with or without treatment. Survival curve analysis was performed using the log-rank test. Results were considered statistically significant at a two-tailed p value of less than 0.05.

3. RESULTS

3.1. Mortality Rates of the Lycopene- and Vehicle-treated Sham-operated and BDL Rats

There was no significant difference in mortality rate between the lycopene- and vehicle-treated (control) BDL rats (control vs. lycopene: 20% (2/10) vs. 10% (1/10), p>0.05). All of the sham-operated rats survived throughout the 4 weeks of treatment.

3.2. Body Weight, Hemodynamics and Biochemistry Parameters of the BDL and Sham-operated Rats With or Without Lycopene Treatment

Table 1 shows the results of the series 1 study including BW, hemodynamic and biochemistry parameters. The rats in the BDL group had a significantly lower BW compared to the sham-operated group [BDL+lycopene (BL) vs. BDL+vehicle (BV) vs. Sham+vehicle (SV): 346 ± 45 vs. 331 ± 57 vs. 416 ± 25 g, respectively; BL and BV vs. SV, $p < 0.05$]. The PP was significantly elevated in the BDL groups compared to the control groups, and it was not significantly reduced by lycopene (BL vs. BV vs. SV: 15.3 ± 3.5 vs. 16.5 ± 3.5 vs. 8.7 ± 0.6 mmHg, respectively; BL and BV vs. SV, $p < 0.05$). The MAP and HR were not significantly different between the BDL and sham groups. The plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin were also significantly elevated in the BDL groups compared to the sham-operated groups, and were not significantly influenced by lycopene (BL vs. BV vs. SV: AST = 576 ± 155 vs. 619 ± 140 vs. 130 ± 55 IU/L; ALT = 114 ± 36 vs. 167 ± 53 vs. 55 ± 12 IU/L; total bilirubin = 8.4 ± 1.1 vs. 8.5 ± 0.7 vs. 0.03 ± 0.01 mg/dL, respectively; BL and BV vs. SV, $p < 0.05$). Lycopene did not affect creatinine levels in the BDL and sham-operated rats.

3.3. Comprehensive Hemodynamic Changes in the Sham-operated and BDL Rats With or Without Lycopene Treatment

Table 2 shows the results of the series 2 study of comprehensive hemodynamic changes in the rats with or without lycopene treatment. The BDL rats had a significantly lower BW compared to the sham-operated rats (BL vs. BV vs. SV: 337 ± 32 vs. 355 ± 27 vs. 427 ± 30 g, respectively; BL and BV vs. SV, $p < 0.05$). In addition, the BDL rats had significantly lower blood pressure, higher PP and lower superior mesentery arterial resistance compared to the sham-operated rats (BL vs. BV vs. SV: MAP = 111 ± 22 vs. 105 ± 12 vs. 137 ± 21 mmHg; PP = 15.4 ± 4.0 vs. 14.0 ± 1.7 vs. 8.8 ± 1.2 mmHg; superior mesentery arterial resistance = 14.0 ± 4.0 vs. 13.7 ± 4.2 vs. 19.9 ± 2.0 mmHg/ml/min/100 g, respectively; BL and BV vs. SV, both $p < 0.05$). A higher CI and lower SVR were also noted in the BDL rats compared to the sham-operated rats (BV vs. SV: CI = 45 ± 9.4 vs. 34 ± 5.9 ml/min/100 g BW; SVR = 2.4 ± 0.5 vs. 4.2 ± 1.2 mmHg/ml/min/100 g BW, respectively), indicating a hyperdynamic circulatory status. The higher cardiac output and CI were significantly decreased by lycopene in the BDL rats (BL vs. BV: cardiac output = 104 ± 27 vs. 159 ± 22 ml/min; CI = 31 ± 7.4 vs. 45 ± 9.4 ml/min/100 g BW, respectively, both $p < 0.05$). Meanwhile, lycopene significantly increased SVR in the BDL rats (3.7 ± 1.0 vs. 2.4 ± 0.5 mmHg/ml/min/100 g BW, respectively, $p < 0.05$).

Table 1

Hemodynamic and biochemistry parameters of the sham-operated and BDL rats with or without lycopene treatment

	BDL + lycopene (BL, n = 9)	BDL + vehicle (BV, n = 8)	Sham + vehicle (SV, n = 9)
BW1 (g)	292 ± 22	283 ± 11	293 ± 15
BW2 (g)	346 ± 45*	331 ± 57*	416 ± 25
MAP (mmHg)	125 ± 18	122 ± 13	135 ± 17
PP (mmHg)	15.3 ± 3.5*	16.5 ± 3.5*	8.7 ± 0.6
HR (beats/min)	379 ± 44	371 ± 48	395 ± 35
AST (IU/L)	576 ± 155*	619 ± 140*	130 ± 55
ALT (IU/L)	114 ± 36*	167 ± 53*	55 ± 12
TB (mg/dL)	8.4 ± 1.1*	8.5 ± 0.7*	0.03 ± 0.01
Cr (mg/dL)	0.53 ± 0.15	0.51 ± 0.19	0.39 ± 0.07

AST = aspartate aminotransferase; ALT = alanine aminotransferase; BW1 = body weight before treatment; BW2 = body weight after different treatments; HR = heart rate; Cr = creatinine; MAP = mean arterial pressure; PP = portal pressure; TB = total bilirubin.
*BL, BV vs. SV, $p < 0.05$.

Table 2

Comprehensive hemodynamic changes in the sham-operated and BDL rats with or without lycopene treatment

	BDL + lycopene (BL, n = 8)	BDL + vehicle (BV, n = 6)	Sham + vehicle (SV, n = 6)
BW (g)	337 ± 32[a]	355 ± 27[a]	427 ± 30
MAP (mmHg)	111 ± 22[a]	105 ± 12[a]	137 ± 21
PP (mmHg)	15.4 ± 4.0[a]	14.0 ± 1.7[a]	8.8 ± 1.2
HR (beats/min)	358 ± 32	361 ± 31	416 ± 41
SMAf (ml/min/100 g)	7.1 ± 1.5	7.0 ± 1.8	6.7 ± 1.4
PVf (ml/min/100 g)	9.7 ± 2.1	11.4 ± 2.1	9.1 ± 1.2
SMAR (mmHg/ml/min/100 g)	14.0 ± 4.0[a]	13.7 ± 4.2[a]	19.9 ± 2.0
CO (ml/min)	104 ± 27[b]	159 ± 22	144 ± 21
CI (ml/min/100 g)	31 ± 7.4[b]	45 ± 9.4[c]	34 ± 5.9
SVR (mmHg/ml/min/100 g)	3.7 ± 1.0[b]	2.4 ± 0.5[c]	4.2 ± 1.2

BW = body weight after different treatments; CO = cardiac output; CI = cardiac index; HR = heart rate; MAP = mean arterial pressure; PP = portal pressure; SMAf = superior mesentery arterial flow; PVf = portal venous flow; SMAR = superior mesentery arterial resistance; SVR = systemic vascular resistance.
[a]BL, BV vs. SV, $p < 0.05$.
[b]BL vs. BV, $p < 0.05$.
[c]BV vs. SV, $p < 0.05$.

3.4. Histopathological Changes, Liver Fibrosis and Intrahepatic Angiogenesis of the Livers in the BDL Rats

Hepatic H&E staining in the BDL rats showed high mononuclear cell infiltration, ballooning change of hepatocytes and bile duct proliferation, indicating inflammatory changes in the livers, which were not significantly influenced by lycopene. Sirius Red staining revealed obvious fibrosis in the livers of the BDL rats. The liver fibrosis was attenuated by lycopene treatment. In addition, there were many CD31-positive stained cells in the livers of the BDL rats, indicating increased intrahepatic angiogenesis. Lycopene reduced the number of positively stained CD31 cells compared to control group (Fig. 1).

3.5. Hepatic Protein Expressions in the BDL Rats

Fig. 2 shows the hepatic protein expressions of the BDL rats treated with lycopene or vehicle (n = 11:8). Lycopene upregulated Akt, eNOS and PI3K, and downregulated VEGFR-2 protein expressions (lycopene vs. control: Akt/β-actin = 0.89 ± 0.17 vs. 0.56 ± 0.17; eNOS/β-actin = 0.72 ± 0.23 vs. 0.21 ± 0.14; PI3K/β-actin = 0.65 ± 0.22 vs. 0.20 ± 0.20; VEGFR-2/β-actin = 0.43 ± 0.19 vs. 0.65 ± 0.15; all $p < 0.05$). ERK (42/44) and iNOS protein expressions were not significantly influenced by lycopene (ERK(42)/β-actin = 0.62 ± 0.16 vs. 0.60 ± 0.15; ERK(44)/β-actin = 0.86 ± 0.16 vs. 0.84 ± 0.09; iNOS/β-actin = 0.60 ± 0.23 vs. 0.49 ± 0.21; all $p > 0.05$).

3.6. Portal-systemic Shunting Degree in the BDL Rats With or Without Lycopene Treatment

Fig. 3 shows the degree of portal-systemic shunting in the vehicle- and lycopene-treated BDL rats (n = 6:6). Lycopene significantly decreased the degree of shunting in the BDL rats.

3.7. Mesenteric Vascular Density in the BDL Rats With or Without Lycopene Treatment

Fig. 4 shows the mesenteric vascular density in the vehicle- and lycopene-treated BDL rats (n = 6:9). Lycopene significantly decreased the mesenteric vascular density in the BDL rats.

4. DISCUSSION

In this study, we showed for the first time that lycopene can reverse portal hypertension-related hyperdynamic circulatory status and ameliorate liver fibrosis and pathological angiogenesis in biliary cirrhotic rats. Consistent with our previous studies, BDL rats presented with jaundice, portal hypertension, and hyperdynamic circulation as evidenced by lower

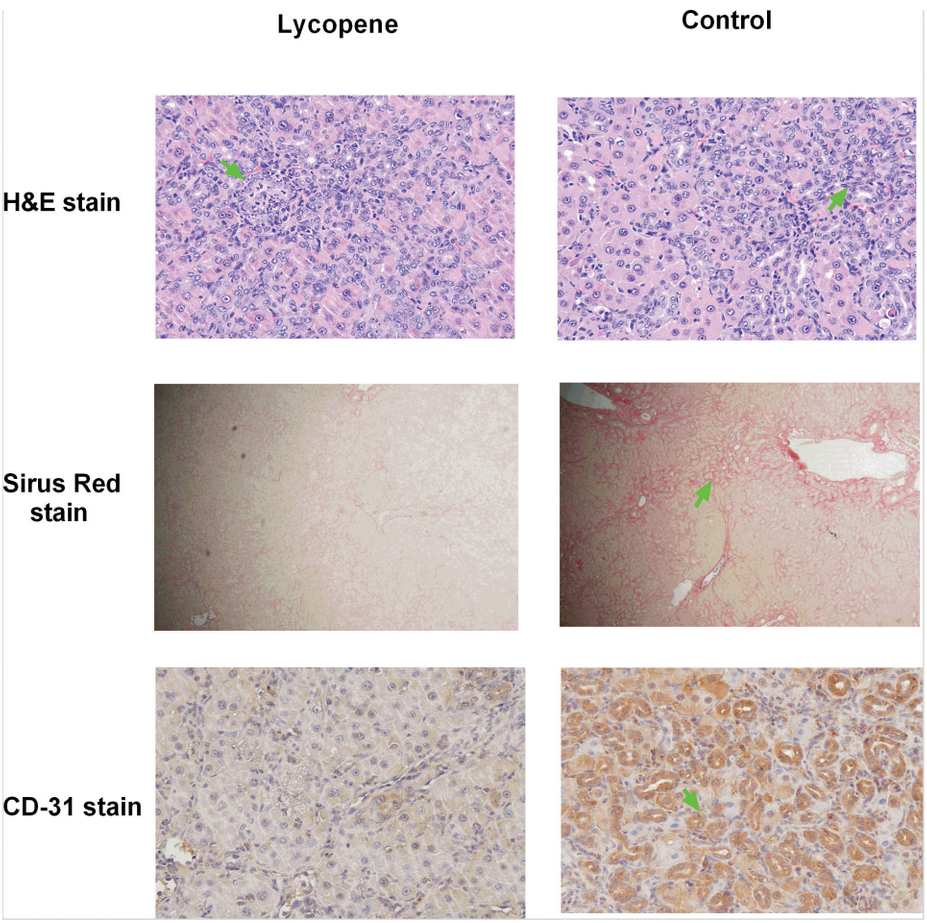

Fig. 1 Liver histology and immunochemical staining of the BDL rats treated with lycopene or vehicle (control). A representative H&E staining image of control and lycopene-treated BDL rats showing ballooning change of hepatocytes accompanied by many inflammatory cells, indicating inflammatory changes in the liver. In the portal area, many inflammatory cells indicated by green arrow were found, and which did not be attenuated by lycopene treatment (magnification 200x, upper panel). Liver fibrosis was demonstrated by Sirius Red staining (magnification 40x, green arrow indicates the fibrotic area, middle panel). Compared with the control group, lycopene attenuated liver fibrosis (middle panel). In addition, many CD31-positive stained cells (green arrow indicating brown cells) were noted in the control group, which was attenuated by lycopene treatment (magnification 200x, lower panel).

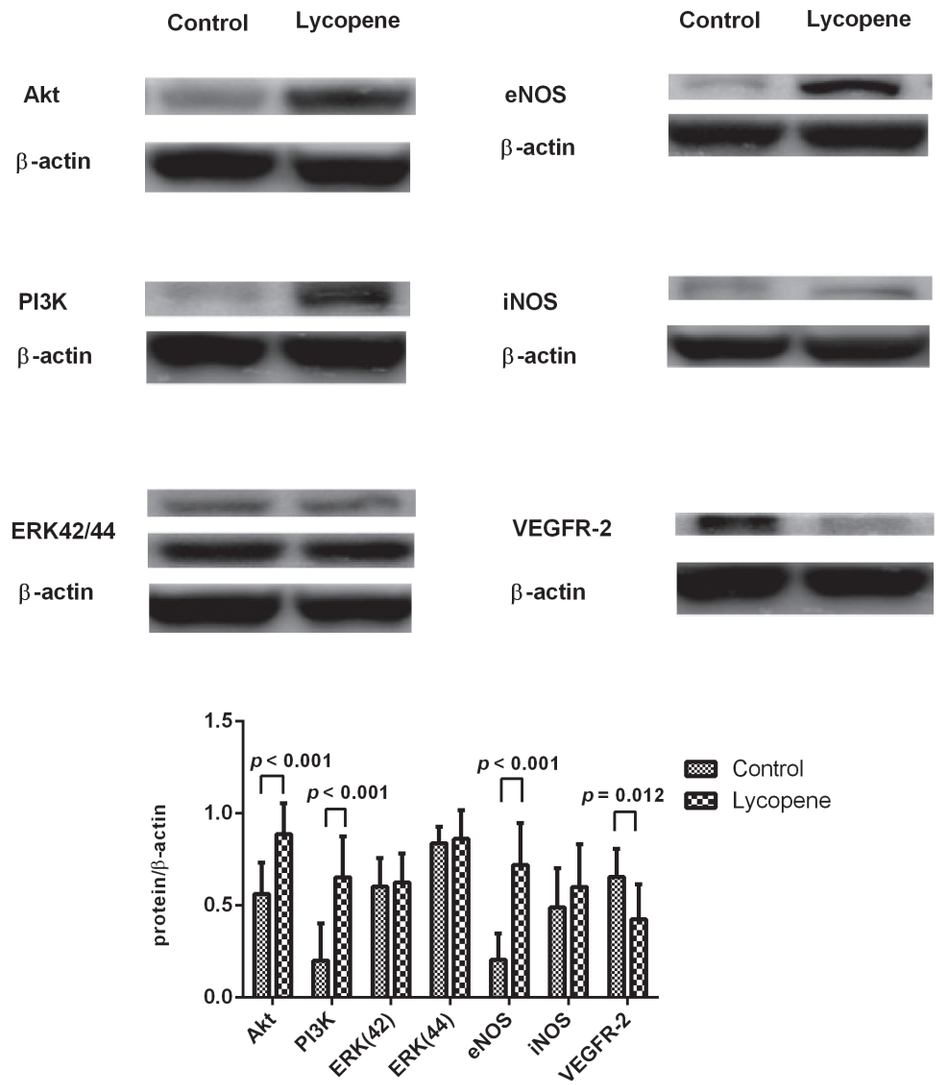

Fig. 2 Hepatic protein expressions of lycopene-treated and control BDL rats. Densitometric quantification and representative Western blots showed that the Akt, eNOS and PI3K expressions were significantly upregulated and the VEGFR-2 protein expression was significantly downregulated by lycopene.

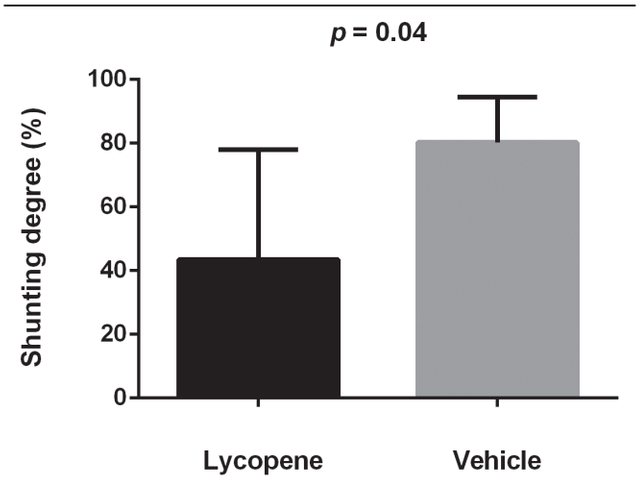

Fig. 3 The portal-systemic collateral shunting degrees of the BDL rats with lycopene or vehicle treatment. Lycopene significantly decreased the shunting degree compared to vehicle in the BDL rats.

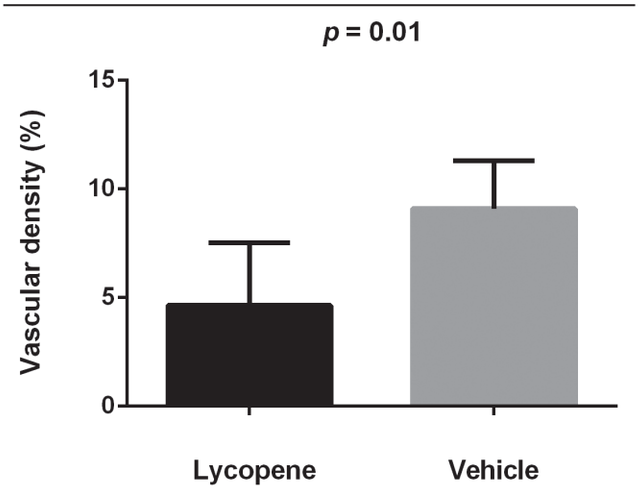

Fig. 4 The effects of lycopene and vehicle treatment on mesenteric vascular density in the BDL rats. Lycopene significantly decreased the mesenteric vascular density compared to vehicle.

MAP, higher CI, and lower SVR compared to sham-operated rats.[3,18] Although lycopene treatment did not reduce PP, it significantly reduced CI and elevated SVR, indicating the reversal of hyperdynamic circulatory status. A possible explanation for the lycopene-induced alleviation of hyperdynamic circulation may be due to an indirect cirrhosis amelioration-related effect or direct cardiovascular effect. Previous studies have demonstrated that lycopene exerted beneficial effects on lowering blood pressure through anti-inflammation and vasodilatation in hypertensive rats;[19,20] however, our data showed that lycopene actually elevated SVR in cirrhotic rats. In addition, a previous study reported that lycopene treatment increased the ejection fraction of the heart in patients with congestive heart failure,[21] whereas we found that lycopene reduced CI, an index of cardiac output, in cirrhotic rats. Therefore, the lycopene-induced reversal of hyperdynamic circulation in the cirrhotic rats seemed to be secondary to improvements in liver cirrhosis rather than a direct systemic cardiovascular effect in our BDL-induced cirrhotic animal model. That is, although the change in PP was not significant, the amelioration of liver cirrhosis led to an improvement in hyperdynamic circulation.

Angiogenesis is critical in the development and maintenance of liver fibrosis, splanchnic hyperemia, and portal-systemic collaterals in portal hypertension.[22–24] In the present study, we showed that 4 weeks of lycopene treatment significantly reduced portal-systemic collaterals and mesenteric vasculature. At the same time, lycopene decreased intrahepatic angiogenesis in the BDL rats as evidenced by a reduction in the number of positively stained CD31 endothelial cells. Western blotting revealed that hepatic eNOS, Akt, and PI3K protein expressions were upregulated whereas the expression of VEGFR-2 protein was downregulated by lycopene treatment. VEGF and its receptor VEGFR-2 have been shown to be overexpressed in cirrhotic livers,[24] and the inhibition of VEGFR-2 has been shown to prevent portal-systemic collateral vessel formation and reverse portal hypertension.[23] Therefore, the downregulation of hepatic VEGFR-2 in the present study may, at least partly, explain the attenuation of intrahepatic angiogenesis. On the other hand, lycopene has been shown to attenuate oxidative stress through activation of the PI3K/Akt pathway.[25] In addition, the effect of lycopene on vascular remodeling through activation of the liver X receptor-PI3K-Akt signaling pathway in mice has been reported.[26] Lycopene has also been shown to attenuate high glucose-mediated apoptosis in podocytes by promoting autophagy via the activation of the PI3K/Akt signaling pathway.[27] Taken together, these findings suggest that in cirrhotic livers, downregulation of VEGFR-2 is the main factor contributing to the reduction in intrahepatic angiogenesis, and that PI3K/Akt/eNOS upregulation may contribute to various beneficial effects such as combating oxidative stress and fibrosis. The expressions of liver inflammation and damage parameters including ALT, AST, and total bilirubin were not influenced by lycopene. This may be because BDL is an overwhelming biliary cirrhosis model, and it is not easy to overcome the inflammation and cholestasis at the end-stage of the model, which was also shown by the H&E staining.

We also observed an anti-fibrotic effect of lycopene in this study. In primary fibroblast cultures from normal peritoneum and adhesion tissues, lycopene has been shown to decrease type I collagen and VEGF mRNA levels in adhesion fibroblasts.[28] Moreover, Kitade *et al.* reported that lycopene suppressed hepatic fibrogenesis in Long-Evans rats, and that this was related to the inhibition of stellate cell activity.[29] A clinical study also revealed that lycopene was efficacious in the management of oral submucosal fibrosis.[30] In the current study, we found that lycopene improved liver fibrosis, as evidenced by Sirius Red staining of the livers. In addition, the protein expressions of PI3K/Akt were upregulated. Similar to our findings, Wu *et al.* reported that quercetin improved liver fibrosis by attenuating hepatic stellate cell activation in BDL rats through the upregulation of the PI3K/Akt pathway.[31] Indeed, cross-talk between liver fibrosis and angiogenesis pathways is complex and may generate different effects depending on the cellular context and biological processes.[32] Therefore, the lycopene-induced downregulation of VEGFR-2 and upregulation of PI3K/Akt may contribute to both anti-angiogenesis and anti-fibrosis in cirrhotic livers. In addition, we also found that the protein expression of eNOS was upregulated. Lycopene has been shown to alleviate vascular arteriosclerosis in allograft transplantation via upregulation of the eNOS pathway.[33] Hepatic eNOS expression was significantly downregulated in cirrhotic status, and the NOS substrate L-arginine upregulated eNOS and ameliorated fibrosis in cirrhotic mice.[33] This suggests that the beneficial effects of L-arginine on liver fibrosis may be related to the maintenance of an appropriate level of eNOS-derived nitric oxide production and thus the restoration of hepatic blood flow, followed by alleviation of liver damage and fibrogenesis.[34] Further studies are warranted to investigate this issue.

The translation of our findings to clinical use should be done with caution with regard to the use of lycopene. The dose of lycopene in clinical studies has varied widely, ranging from 1.44 to 75 mg per day, making it difficult to establish the optimal level.[35] In animal studies, the dose of lycopene has ranged from 2.5 to 20 mg/kg/day, since rodents have 10% of human absorption.[36–38] To achieve a 15 mg dose of lycopene through dietary consumption in humans, it is necessary to consume about 3–4 whole tomatoes, or 2 tablespoons of tomato paste daily, a quantity that may not be feasible for some people.[35] Therefore, lycopene supplementation may be a more realistic approach to achieve a beneficial level with respect to ameliorating liver fibrosis in cirrhotic patients.

In conclusion, lycopene reversed hyperdynamic circulation, ameliorated liver fibrosis, and reduced abnormal intra- and extra-hepatic angiogenesis without influencing liver and renal biochemistry parameters in the cirrhotic rats in this study. The possible mechanism may be due to various effects of VEGFR2/PI3K/Akt/eNOS pathways. Further studies for the possible clinical application of lycopene in cirrhotic patients are warranted.

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