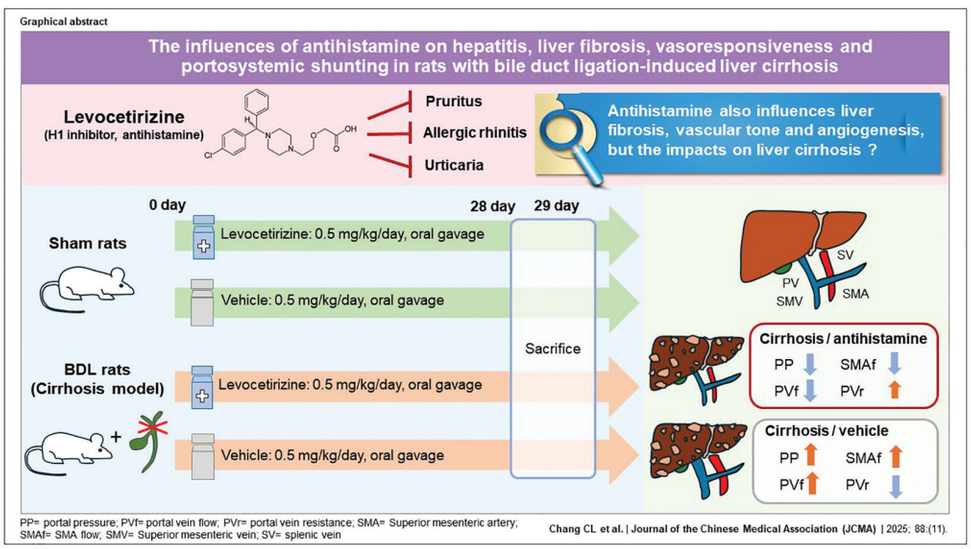
J Chin Med Assoc

ORIGINAL ARTICLE

The influences of antihistamine on liver fibrosis, vasoresponsiveness, and portosystemic shunting in bile duct–ligated cirrhotic rats

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Abstract

Background: Liver cirrhosis with portal hypertension is a sequela of chronic hepatitis, characterized by liver fibrosis, poor portosystemic collateral vasoresponsiveness, increased mesenteric angiogenesis, and collateral formation. Antihistamines are frequently used to control pruritus in cirrhotic patients, and it has also been shown to reverse histamine-induced inflammation, fibrogenesis, angiogenesis, and vasodilatation. However, the relevant impacts on cirrhosis are not clear.

Methods: Liver cirrhosis was induced in rats with common bile duct ligation (BDL). Sham rats were surgical controls. Levocetirizine (0.5 mg/kg/day, oral gavage) or vehicle was administered from the 1st day after operations and experiments were performed on the 29th day to survey: (a) Systemic and splanchnic hemodynamic parameters; (b) serum liver and renal biochemistry parameters; (c) protein expressions of liver fibrogenesis factors; (d) portosystemic collateral vasoresponsiveness to vasopressin; (e) portosystemic shunting degree; and (f) mesenteric angiogenesis.

Results: Compared with the vehicle, levocetirizine decreased portal pressure, superior mesenteric artery flow, portal vein (PV) flow, and elevated PV resistance in BDL rats. Levocetirizine significantly down-regulated the hepatic TGF-β, p-Smad2, MMP-2, and TIMP-1 protein expressions in BDL rats. Levocetirizine did not affect the collateral vasoresponsiveness to vasopressin but tended to reduce the severity of portosystemic shunting and mesenteric angiogenesis.

Conclusion: Levocetirizine ameliorated liver fibrosis, portal hypertension, and splanchnic hyperdynamic circulation in cirrhotic rats, possibly through the downregulation of hepatic fibrogenesis factors. Antihistamines may be a therapeutic option to control portal hypertension. However, further investigations are required to clarify its clinical application.

Keywords: Angiogenesis; Antihistamine; Liver cirrhosis; Portal hypertension

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Lay Summary: Histamine receptor one antagonist (antihistamine) is frequently used to control allergy symptoms and is also applied to control pruritus in cirrhotic patients. Interestingly, antihistamine also confers vascular and antifibrotic actions, but the relevant impacts on cirrhosis and portal hypertension have not been surveyed thoroughly. The current study identifies that the antihistamine levocetirizine reduces portal pressure and alleviates splanchnic hyperdynamic circulation in cirrhotic rats, which may be linked to liver fibrogenesis factors downregulation and reduced intrahepatic resistance. Antihistamines may be applied to control portal hypertension and further clinical investigation is anticipated.

1. INTRODUCTION

Acute and chronic liver injuries are usually followed by different kinds of tissue repair. Unfortunately, they may not be able to resume the normal liver microenvironment, and on the contrary, fibrogenesis may ensue, which leads to liver cirrhosis with elevated portal pressure, the so-called portal hypertension. The increased intrahepatic resistance can be attributed to structural changes, including the formation of regeneration nodules and collagen fiber deposition. The difficulty of portal blood flow to enter the liver triggers the formation of portosystemic collaterals, among which gastroesophageal variceal hemorrhage can be fatal in clinical settings. Furthermore, animal studies have proved that the vasoresponsiveness to splanchnic vasoconstrictors such as terlipressin is poorer in portal hypertension with acute hemorrhage,[1] which may adversely affect the therapeutic efficacy of variceal hemorrhage. Recently, angiogenesis has also been proposed to participate in the mechanism of portal hypertension and collateral formation.

Pruritus is an annoying condition in patients with liver diseases and is usually alleviated by an antihistamine that acts against the type 1 histamine receptor (H_1 antagonist). Interestingly, histamine exerts fibrogenesis, angiogenesis, and vasoactive effects, which raises the concern whether antihistamine influences the severity of fibrosis, angiogenesis, and vascular tones in liver cirrhosis and portal hypertension, but these aspects have not been surveyed thoroughly. The previous studies have identified that histamine stimulates collagen synthesis and fibroblast proliferation in abnormal scars.[2] Another study indicated that histamine released by mast cells after injuries in the peritoneal surface caused vasodilatation and protein-enriched exudate. These exudates constituted the base of fibrosis and adhesion of the wound.[3] Consistent with these notions, an antihistamine ameliorated peritoneal adhesion.[4]

Regarding the intra-abdominal vascular effects of histamine, the release of histamine from intestinal mast cells has been implicated in splanchnic vasodilatation and postprandial hyperemia.[5] It is worth noting that postprandial hyperemia is a significant contributing factor to variceal hemorrhage.[6] Histamine induces endothelium-dependent relaxation by stimulation of H_1 receptors in the mesenteric arterial bed.[7] High concentrations of histamine (10^{-3}-≥ 10^{-1} M) induced relaxation of the isolated longitudinal rat portal vein as well.[8] This is supported by another on coronary vasculature, showing that histamine evoked vasodilations through activating H_1 receptor.[9] However, contradictory results suggested that activation of the H_1 receptor induced contraction of the mouse basilar artery.[10]

Taking the relationship between histamine and angiogenesis into consideration, histamine is produced in rapidly growing tissues, and has been suggested to promote neoplastic growth and angiogenesis.[11] Mast-cell granule histamine is mitogenic to human microvascular endothelial cells, which could be reproduced by H_1-agonist and inhibited by H_1-antagonist.[12] Histamine is also reported as an angiogenic factor in vivo: Zauberman et al[13] found that exogenous histamine released from tubes inserted in the rabbit cornea stimulated corneal angiogenesis. It has been demonstrated that systemic treatment with clemastine, a H_1 antagonist, suppressed mast-cell-mediated angiogenesis by hampering the microvascular spatial expansion, as assessed by the rat mesenteric window assay.[14] It has been identified that epinastine hydrochloride, a selective H_1 antagonist, significantly inhibited the production of vascular endothelial growth factor (VEGF) in murine mast cells.[15]

Antihistamine is frequently used to alleviate pruritus in patients with liver diseases. There has also been accumulating evidence indicating the remarkable anti-fibrosis, anti-angiogenesis actions, and vasoactive properties of antihistamines in different research fields. However, the relevant survey on chronic liver injury with subsequent liver cirrhosis and portal hypertension has not been performed. This study, therefore, aimed to investigate whether antihistamine modulates the aforementioned aspects in rats with common bile duct ligation (BDL)-induced liver cirrhosis.

2. METHODS

2.1. Animal model

Male Sprague-Dawley rats weighing 240 to 270 g were used. They were caged at 24°C, with a 12-hour light-dark cycle and free access to food and water until the time of experiments. Survival surgery and hemodynamic study were performed under anesthesia with Zoletil intramuscular injection (0.8 mg/kg). All experimental procedures were conducted in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, Guidance on the operation of the Animals (Scientific Procedures) Act 1986, and the principles outlined in the Guide for the Care and Use of Laboratory Animals (8th ed., 2011. Institute for Laboratory Animal Research, The National Academies Press, Washington, D.C., USA). The animal experiments had been approved by the Animal Experiments Committees of Taipei Veterans General Hospital (IACUC 2016-85) and Chang Gung Memorial Hospital (2015121810), respectively.

2.2. Common BDL

Secondary biliary cirrhosis was induced with BDL as previously described.[16] Under Zoletil anesthesia, the common bile duct was exposed through a midline abdominal incision, catheterized by a PE-10 catheter, and doubly ligated with 3-0 silk. The first ligature was made below the junction of the hepatic ducts, and the second ligature above the entrance of the pancreatic

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Conflicts of interest: Dr. Ching-Chih Chang, Dr. Ming-Chih Hou, and Dr. Fa-Yauh Lee, editorial board members of the Journal of the Chinese Medical Association, had no role in the peer review process or in the decision to publish this article. The other authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Author contributions: Dr. Shao-Jung Hsu and Dr. Ming-Hung Tsai contributed equally to this work.

Journal of Chinese Medical Association. (2025): 825-835.

Received August 3, 2025; accepted September 1, 2025.

doi: 10.1097/JCMA.0000000000001301

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duct. The PE-10 catheter was then removed and the ligatures tightened, followed by the sectioning of the common bile duct between the ligatures. A high yield of secondary biliary cirrhosis was noted four weeks after the ligation.[17] To avoid coagulation defects, BDL rats received weekly vitamin K injection (50 μg/kg intramuscularly).[17]

2.3. Measurement of systemic and portal hemodynamics

The right femoral artery was cannulated with a PE-50 catheter that was connected to a Spectramed DTX transducer (Spectramed Inc., Oxnard, CA). Continuous recordings of mean arterial pressure (MAP), heart rate (HR), and portal pressure (PP) were performed on a multi-channel recorder (model RS 3400, Gould Inc., Cupertino, CA). The external zero reference was placed at the level of the mid-portion of the rat. The abdomen was opened with a midline incision, and a mesenteric vein was cannulated with a PE-50 catheter connected to a Spectramed DTX transducer. Then the abdominal cavity was closed, and the PP was recorded on a Gould model RS 3400 recorder.[18]

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 (T206 small animal blood flow metre, Transonic system Inc., Ithaca, NY) was placed to measure the flow. The measurement of portal vein (PV) flow was performed with similar steps.

Cardiac output (CO) was measured by thermodilution, as previously described.[16] Cardiac index (CI, mL/min/100 g BW) was calculated as CO per 100 g BW. Stroke volume (SV) was calculated as CO divided by HR (mL/beats). Systemic vascular resistance (SVR, mmHg/mL/min/100 g BW) was calculated by dividing MAP by CI. SMA (or PV) resistance (SMAR or PVR) (mmHg/mL/min/100 g BW) was calculated by (MAP-PP)/SMA (or PV) flow per 100 g BW.

2.4. Hepatic fibrosis determination with Sirius red staining

Liver paraffin sections underwent staining using a Sirius red staining kit (Polysciences Inc., Warrington, PA). Analysis of the proportion of the area stained with Sirius red was performed using ImageJ software. This entailed converting the image to grayscale, then isolated the collagen-stained region in red by applying the thresholding function. The quantified area meeting the threshold criteria was then calculated and expressed as a percentage of the total image area.

2.5. Western blot

The liver protein was extracted by pulverization with liquid nitrogen, then a ratio of 1 mL of lysis buffer (phosphate-buffered solution containing 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), and 0.05% protease inhibitor cocktail solution (Roche Diagnostics GmbH, Penzberg, Germany) for each 100 mg powdered liver sample was used. Protein concentration was determined for each sample by the Bradford method.[19] An aliquot of 20 to 40 μg protein from each sample that dissolved in sample buffer (63 mmol/L of Tris-HCL, pH 6.8, containing 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.005% bromophenol blue) and 10 μg positive control were separated on denaturing SDS-10% polyacrylamide gels by electrophoresis (Mini-PROTEAN® 3 Cell, Bio-Rad Laboratories, Hercules, CA). Prestained protein markers (SDS-PAGE Standards, Bio-Rad Laboratories, Hercules, CA) were used for molecular weight determinations. The primary antibodies were α-SMA (GeneTex, Irvine, CA, GTX100034, 1:3000), β-actin (GeneTex, GTX629630, 1:5000), collagen-1 (Proteintech, Rosemont, IL, 14695-1-AP, 1:1000), MMP-2 (GeneTex, GTX30147, 1:1000), p-SMAD2 (Cell Signaling, Danvers, MA, 3108S, 1:1000), SMAD2 (GeneTex, GTX111075, 1:1000), TGF-β (GeneTex, GTX45121, 1:1000), TIMP-1 (abcam, Waltham, MA, ab61224, 1:2000). Proteins were then transferred to a polyvinylidene difluoride membrane (Immun-BlotTM PVDF Membrane, Bio-Rad Laboratories, Hercules, CA) by a semi-dry electroblotting system (Trans-Blot® SD Semi-dry Electrophoretic Transfer Cell, Bio-Rad Laboratories, Hercules, CA). To block nonspecific binding, membranes were blocked with 3% nonfat dry milk in TBS-T, pH 7.4 (25 mmol/L Tris base-137 mmol/L NaCl-2.7 mmol/L KCL-1% Tween 20). Blots were incubated with the primary antibody diluted with 3% nonfat dry milk in TBS-T, then washed. After that, the blots were incubated with the secondary antibody diluted with 3% nonfat dry milk in TBS-T and washed. Specific proteins were detected by enhanced chemiluminescence (Immobilon Western Chemiluminescent HRP Substrate, Merk Millipore Co., Billerica, MA) and scanned with a computer-assisted video densitometer and digitalization system (BioSpectrum® 600 Imaging System, Ultra-Violet Products Ltd., Upland, CA). Signal intensity (integral volume) of the appropriate band was then analyzed.

2.6. In situ portosystemic collateral perfusion

The in situ perfusion system was performed as previously described.[20] Briefly, both jugular veins were cannulated with 16-gauge Teflon cannulas, and an 18-gauge Teflon cannula was inserted in the distal SMV. To exclude the liver from perfusion, the second loose ligature around the PV was tied. Experiments were performed 15 minutes after starting perfusion, at a constant rate of 12 mL/min. In each individual preparation, after testing experimental agents, the contracting capability of the portosystemic collateral vessels was challenged with a 125-mM potassium chloride solution.

2.7. Color microsphere method for portosystemic shunting degree analysis

Portosystemic shunting degree was determined according to the technique introduced by Chojkier[21] except substituting color for radioactive microspheres. In brief, 30 000 of 15-μm yellow microspheres (Dye Track; Triton Technology, San Diego, CA) were injected into the spleen slowly. The rats were then euthanized, and the livers and lungs were dissected. The number of microspheres in each tissue was determined following the protocol provided by the manufacturer, and 3000 blue microspheres served as the internal control. The tissue was digested overnight with 1 M KOH at 60°C and thoroughly sonicated. After centrifugation, the supernatant was removed, and the pellets were washed once with 10% Triton X-100 and twice with acidified ethanol. After that, pellets containing the microspheres were allowed to dry overnight. The microspheres were diluted with 200 μL of acidified Cellosolve acetate (Spectrum Chemicals, Gardena, CA). The absorbance of the solution was read at 448-nm wavelength (yellow) and 670-nm wavelength (blue) by a spectrophotometer (Shimadzu, Columbia, MD), and the number of microspheres was calculated according to standards. The portosystemic shunting was calculated as (number of microspheres) lung/(liver + lung). Assuming a worst-case scenario in which two-thirds of the microspheres remain trapped in the spleen, this technique detects a minimal shunt of 3.5%. Studies using color microspheres have been validated to provide results similar to those using radioactive microspheres.[22]

2.8. Immunofluorescent study for the mesenteric vascular density

Mesenteric angiogenesis was quantified by CD31-labeled microvascular networks in rat mesenteric connective tissue windows according to the previous study with minor modification.[23] From each rat, at least four mesenteric windows

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were dissected free, washed in PBS, dried on gelatin-coated slides, and fixed in 100% MeOH (-20°C for 30 min). Slides were then incubated overnight at 4°C with the primary antibody mouse anti-rat CD31-biotin (AbD Serotec, Oxford, UK). Then, the secondary antibody (CY2-conjugated streptavidin; Jackson ImmunoResearch, West Grove, PA) was applied for 1 hour at room temperature. At least four sets of data were obtained for each mesenteric window. Immunofluorescent images (×100 magnification) were captured using an upright fluorescent microscope (AX80, Olympus, Japan) with charge-coupled device (QICAM, High-performance IEEE 1394 FireWireTM Digital CCD Camera, QImaging, BD, Canada) and thresholded by ImageJ software (available for download from the National Institutes of Health [https://imagej.net/ij/]). The vascular length was manually measured with the pencil tool and the vascular area automatically with histogram function, respectively.

2.9. Drugs

Levocetirizine and arginine vasopressin (AVP) were purchased from Merck KGaA (Darmstadt, Germany). All agents were freshly prepared on the days of the experiment.

2.10. Statistical analysis

All results are expressed as mean ± standard error of the mean Statistical analyses were performed using one-way analysis of variance (ANOVA) with LSD test. Results were considered statistically significant at a two-tailed p value of <0.05.

2.11. Study protocol

Levocetirizine, a peripheral histamine H1 receptor antagonist that lacks central effects[24] at the dose of 0.5 mg/kg/day (distilled water as the vehicle),[25] was administered via oral gavage to sham or BDL rats since the 1st day throughout the 28th day after BDL, and the following parameters were evaluated on the 29th day of treatments, respectively:

a. Hemodynamic parameters measurements: MAP, HR, PP, CO, SVR, SMA flow, PV flow.
b. Serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, blood urea nitrogen (BUN), and creatinine (Cr).
c. Determination of the severity of liver fibrosis with Sirius red staining.
d. Western analyses of hepatic fibrogenesis factors' expressions.

Parallel experimental groups were also applied for:

e. Evaluation of the concentration-response curves to graded concentrations of AVP (10^{-10}, 10^{-9}, 3×10^{-9}, 10^{-8}, 3×10^{-8}, and 10^{-7} M) with an in situ collateral perfusion model.
f. Portosystemic shunting degree determination with the color microsphere method.
g. Mesenteric angiogenesis survey with CD31 immunofluorescence staining of mesenteric windows.

3. RESULTS

3.1. Systemic hemodynamics

Fig. 1 shows the systemic hemodynamic parameters, including MAP, HR, CI, CO, SV, and SVR in sham and BDL rats with vehicle or levocetirizine administration (sham-vehicle, SV, n = 6; sham-levocetirizine, SL, n = 6; BDL-vehicle, BV, n = 7; BDL-levocetirizine, BL, n = 7) for 28 days. As compared with sham rats treated with vehicle, vehicle-administered BDL rats had significantly lower MAP (SV vs BV: 134 ± 4 vs 118 ± 5 mmHg), SVR (4.92 ± 0.30 vs 3.31 ± 0.20 mmHg/mL/min/100 g), and higher CI (27.75 ± 1.45 vs 35.98 ± 1.50 mL/min/100 g), compatible with the development of systemic hyperdynamic circulatory status ($*p < 0.05$, $**p < 0.005$, $***p < 0.001$). As compared with the vehicle, levocetirizine did not significantly influence the aforementioned parameters, either in sham or BDL groups ($p > 0.05$).

3.2. Splanchnic hemodynamics

Fig. 2 shows the body weight (BW) and splanchnic hemodynamic parameters, including PP, SMA flow, SMAR, PV flow, and PVR in sham and BDL rats with vehicle or levocetirizine administration for 28 days. Compared with vehicle-treated sham rats, vehicle-administered BDL rats had lower BW (SV vs BV: 410.5 ± 5.4 vs 361.1 ± 9.0 g), SMAR (22.71 ± 2.38 vs 13.09 ± 1.31 mmHg/mL/min/100 g), PVR (20.52 ± 2.21 vs 9.99 ± 1.42 mmHg/mL/min/100 g) and higher PP (9.2 ± 0.2 vs 17.4 ± 0.5 mmHg), SMA flow (5.7 ± 0.5 vs 8.0 ± 0.7 mL/min/100 g) and PV flow (6.5 ± 0.7 vs 11.4 ± 1.5 mL/min/100 g), compatible with the development of portal hypertension and splanchnic hyperemia ($*p < 0.05$, $**p < 0.005$, $***p < 0.001$). Furthermore, compared with vehicle-treated groups, levocetirizine significantly reduced PP (BV vs BL: 17.4 ± 0.5 vs 16.1 ± 0.6 mmHg), SMA flow (8.0 ± 0.7 vs 6.6 ± 0.2 mL/min/100 g), PV flow (11.4 ± 1.5 vs 7.7 ± 1.0 mL/min/100 g), and increased PVR (9.99 ± 1.42 vs 16.76 ± 2.37 mmHg/mL/min/100 g) in BDL rats.

3.3. Liver and kidney biochemistry parameters

Fig. 3 depicts the liver and kidney biochemistry parameters, including ALT, AST, total bilirubin, BUN, and creatinine in sham and BDL rats treated with vehicle or levocetirizine. BDL rats had significantly higher ALT (SV vs BV: 48.3 ± 3.9 vs 129.5 ± 13.5 U/L), AST (86.1 ± 3.5 vs 699.0 ± 152.2 U/L), total bilirubin (0.02 ± 0.00 vs 8.33 ± 1.01 mg/dL), BUN (20.4 ± 0.7 vs 22.8 ± 1.2 mg/dL) and creatinine (0.37 ± 0.02 vs 0.43 ± 0.04 mg/dL) levels as compared to sham rats with vehicle treatment ($*p < 0.05$, $**p < 0.005$, $***p < 0.001$). Levocetirizine did not influence the aforementioned parameters in sham or BDL rats ($p > 0.05$).

3.4. Severity of hepatic fibrosis

Fig. 4 reveals that the BDL rats (BV: 18.23 ± 1.58, BL: 10.89 ± 0.42) had significantly increased Sirius red-stained area percentage (%, stained area/total area) as compared to the sham rats (SV: 0.44 ± 0.03, SL: 0.40 ± 0.08, $***p < 0.001$ respectively), demonstrating that BDL rats developed liver cirrhosis with increased collagen fiber deposition. Levocetirizine did not influence the stained area percentage in sham rats (SV vs SL, $p > 0.05$) but significantly decreased it in BDL rats (BV vs BL, $p < 0.001$), indicating ameliorated liver fibrosis by levocetirizine treatment.

3.5. Hepatic fibrogenic protein expressions

Fig. 5 shows the hepatic protein expressions of tissue growth factor (TGF)-β, p-Smad2, matrix metalloproteinases (MMP)-2, tissue inhibitor of metalloproteinases (TIMP)-1, smooth muscle actin (α-SMA), collagen-1 in sham and BDL rats with vehicle or levocetirizine administration for 28 days. As compared with vehicle, levocetirizine significantly down-regulated TGF-β (BV vs BL: 1.0831 ± 0.0482 vs 0.6994 ± 0.0878 [/β-actin]), p-Smad2/Smad2 (1.2129 ± 0.0598 vs 0.8693 ± 0.0354), MMP-2 (0.9291 ± 0.0577 vs 0.6665 ± 0.0515 [/β-actin]), TIMP-1 (0.9470 ± 0.0423 vs 0.6191 ± 0.1236 [/β-actin]) expressions ($*p < 0.05$, $**p < 0.005$). The whole blots are shown in the Supplementary Figure, https://links.lww.com/JCMA/A354.

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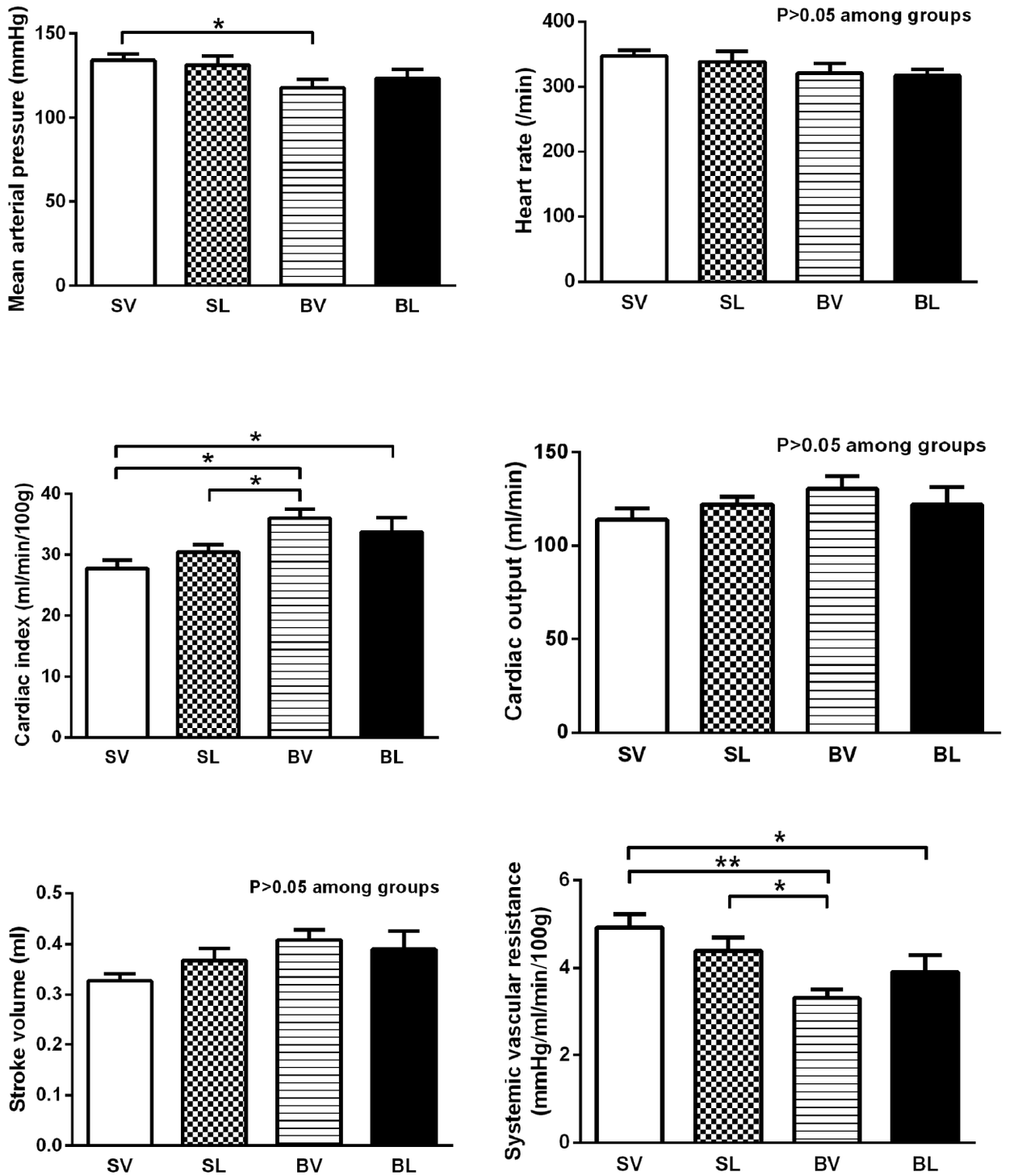


Fig. 1 The systemic hemodynamic parameters, including MAP, HR, CI, CO, SV, and SVR in sham and BDL rats with vehicle or levocetirizine administration. As compared with sham rats treated with vehicle, vehicle-administered BDL rats had significantly lower MAP and SVR and higher CI, compatible with the development of systemic hyperdynamic circulatory status (*$p < 0.05$, **$p < 0.005$). As compared with the vehicle, levocetirizine did not significantly influence the aforementioned parameters, either in sham or BDL groups ($p > 0.05$). BDL = bile duct ligation; BV = BDL-vehicle; CI = cardiac index; CO = cardiac output; HR = heart rate; MAP = mean arterial pressure; SL = sham-levocetirizine; SV = stroke volume; SVR = systemic vascular resistance.

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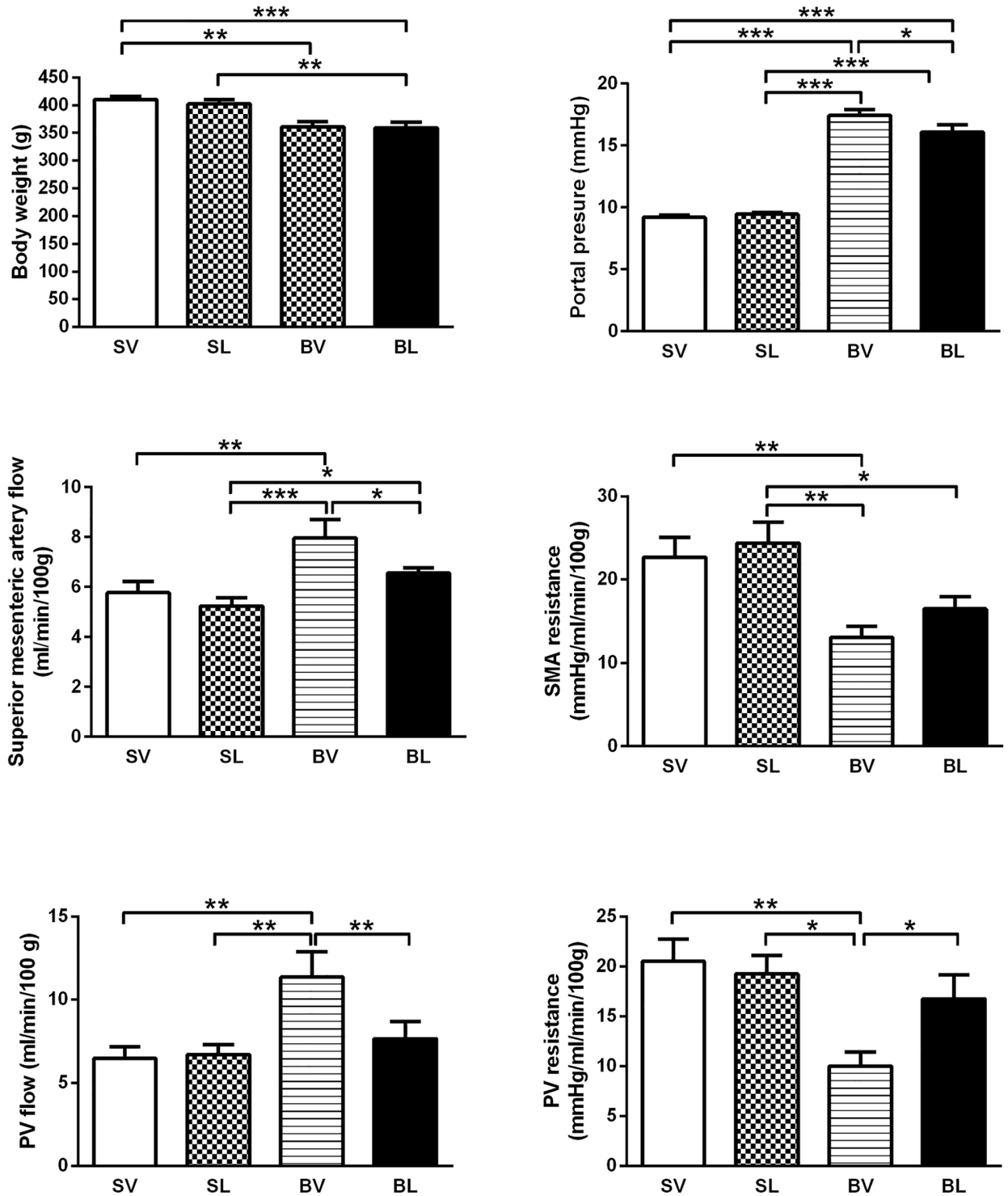


Fig. 2 The BW and splanchnic hemodynamic parameters, including PP, SMA flow, SMAR, PV flow, and PVR in sham and BDL rats with vehicle or levocetirizine administration. Compared with vehicle-treated sham rats, vehicle-administered BDL rats had lower BW, SMAR, and PVR, and higher PP, SMA flow, and PV flow, compatible with the development of portal hypertension and splanchnic hyperemia. Furthermore, compared with vehicle-treated groups, levocetirizine significantly reduced PP, SMA flow, PV flow, and increased PVR in BDL rats (*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$). BDL = bile duct ligation; BV = BDL-vehicle; BW = body weight; PP = portal pressure; PV = portal vein; SL = sham-levocetirizine; SMA = Superior mesenteric artery; SMAR = SMA resistance.

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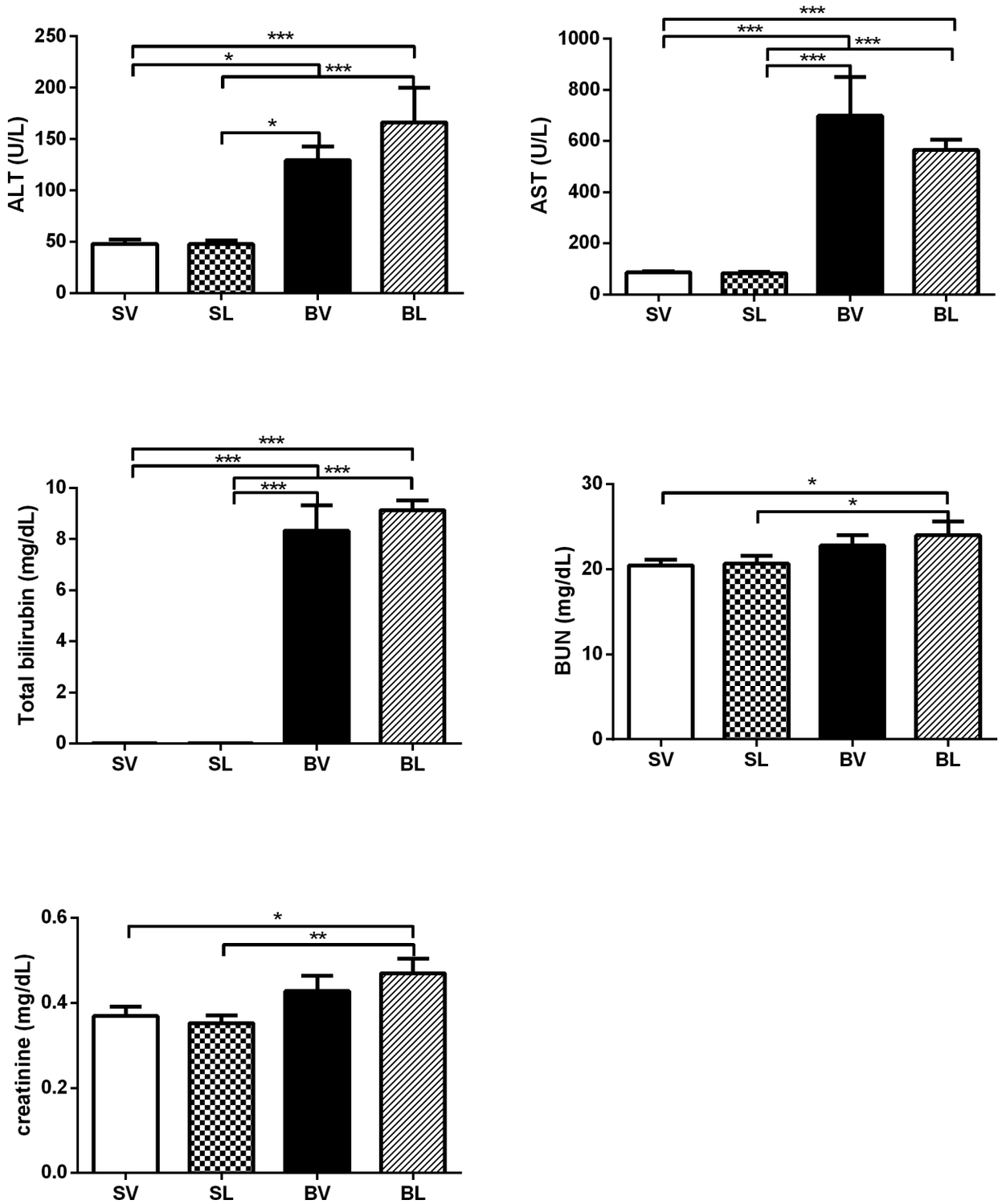


Fig. 3 The liver and kidney biochemistry parameters, including ALT, AST, total bilirubin, BUN, and creatinine in sham and BDL rats treated with vehicle or levocetirizine. Although BDL cirrhotic rats (BV, BL) had significantly higher ALT, AST, total bilirubin, BUN, creatinine levels as compared with the sham rats (SV, SL) (*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$), levocetirizine did not significantly influence these biochemistry parameters in sham or BDL rats. ALT = alanine aminotransferase; BDL = bile duct ligation; BL = BDL-levocetirizine; BUN = blood urea nitrogen; PV = portal vein; SL = sham-levocetirizine.

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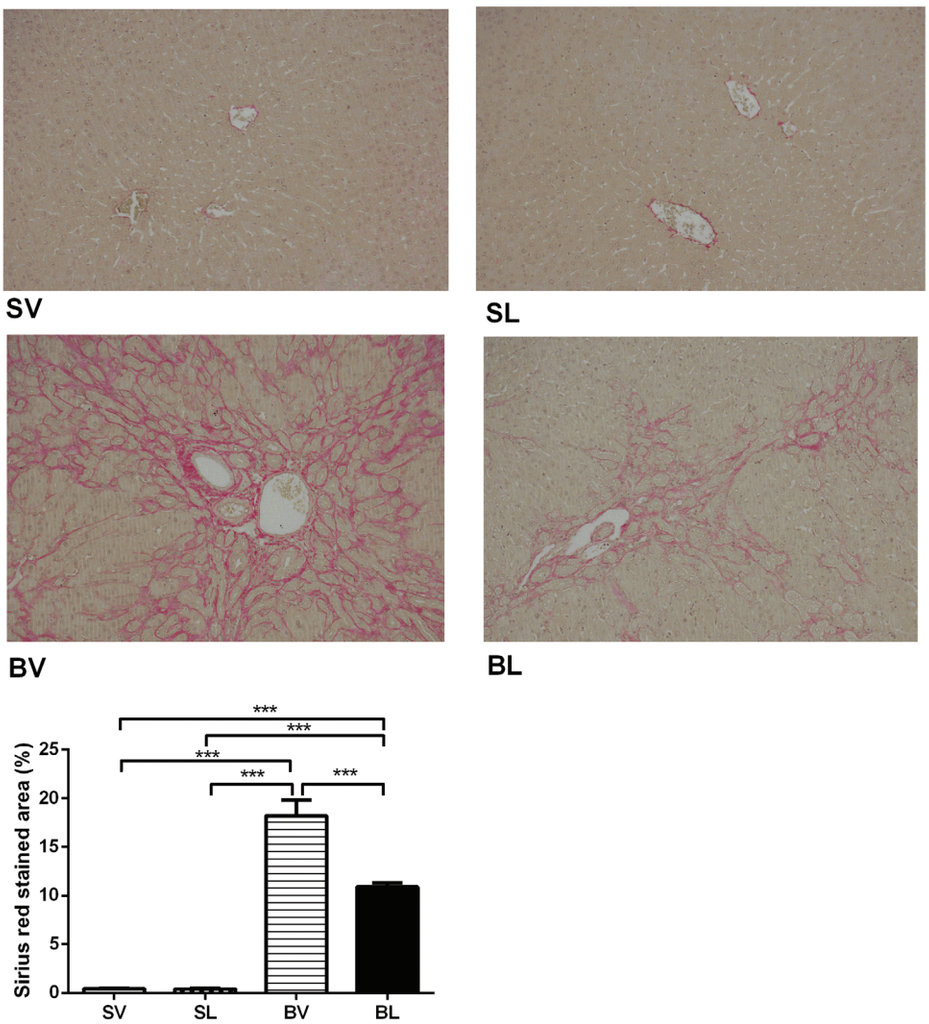


Fig. 4 Representative Sirius red-stained image and area percentage (%, stained area/total area) in sham and BDL rats treated with vehicle or levocetirizine (magnification 40×). The BDL rats had significantly increased Sirius red-stained area percentage as compared to the sham rats ($p < 0.001$, respectively). Levocetirizine did not influence the stained area percentage in sham rats but significantly decreased it in BDL rats (BV vs BL, $p < 0.001$). BDL = bile duct ligation; BV = BDL-vehicle.

3.6. Portosystemic collateral vascular responsiveness to AVP

Fig. 6A depicts the portosystemic collateral vascular bed concentration-response relationship to AVP in BDL rats treated with vehicle or levocetirizine for 28 days. There were no significant changes in perfusion pressures between groups in response to different concentrations of AVP (all $p > 0.05$).

3.7. Shunting degree

Fig. 6B reveals the portosystemic shunting degree in BDL rats treated with vehicle or levocetirizine for 28 days. Although levocetirizine tended to reduce the shunting degree in BDL rats, the statistical significance was not reached (BS vs BL: 72.3% ± 4.1% vs 49.4% ± 10.7%, $p > 0.05$).

3.8. Mesenteric window vascular density

Fig. 6C shows the mesenteric window vascular area in BDL rats treated with vehicle or levocetirizine. Levocetirizine tended to reduce the vascular density in BDL rats, but the statistical significance was not reached (BV vs BL: 82.19 ± 5.75 vs 63.99 ± 9.08 (vascular area/total mesenteric window area), $p > 0.05$).

4. DISCUSSION

The main findings in the current study are that levocetirizine, a histamine H1 receptor blocker that is frequently used to alleviate pruritus in cirrhotic patients, is capable of reducing liver fibrosis, portal pressure, SMA flow, PV flow, and increasing PV resistance without significantly affecting portosystemic collateral vasoresponsiveness, shunting degree, mesenteric angiogenesis, and liver/kidney biochemistry parameters. PP is influenced by three major factors: intrahepatic resistance (the more advanced fibrosis and the higher the intrahepatic vasoconstriction, the higher the resistance), splanchnic blood flow indexed by SMA blood flow, and portosystemic collateral vascular resistance. In brief, the higher the intrahepatic resistance and the higher the SMA blood flow, the higher the PP. Because the portosystemic collateral vascular responsiveness to AVP, shunting degree, and mesenteric angiogenesis were not affected by levocetirizine, the net results suggest that levocetirizine ameliorates portal hypertension mainly via alleviating liver fibrosis and reducing intrahepatic resistance. This is supported by the finding that the hepatic fibrogenesis factors protein expressions, including TGF-β, p-Smad2, and MMP-2, were down-regulated. Furthermore,

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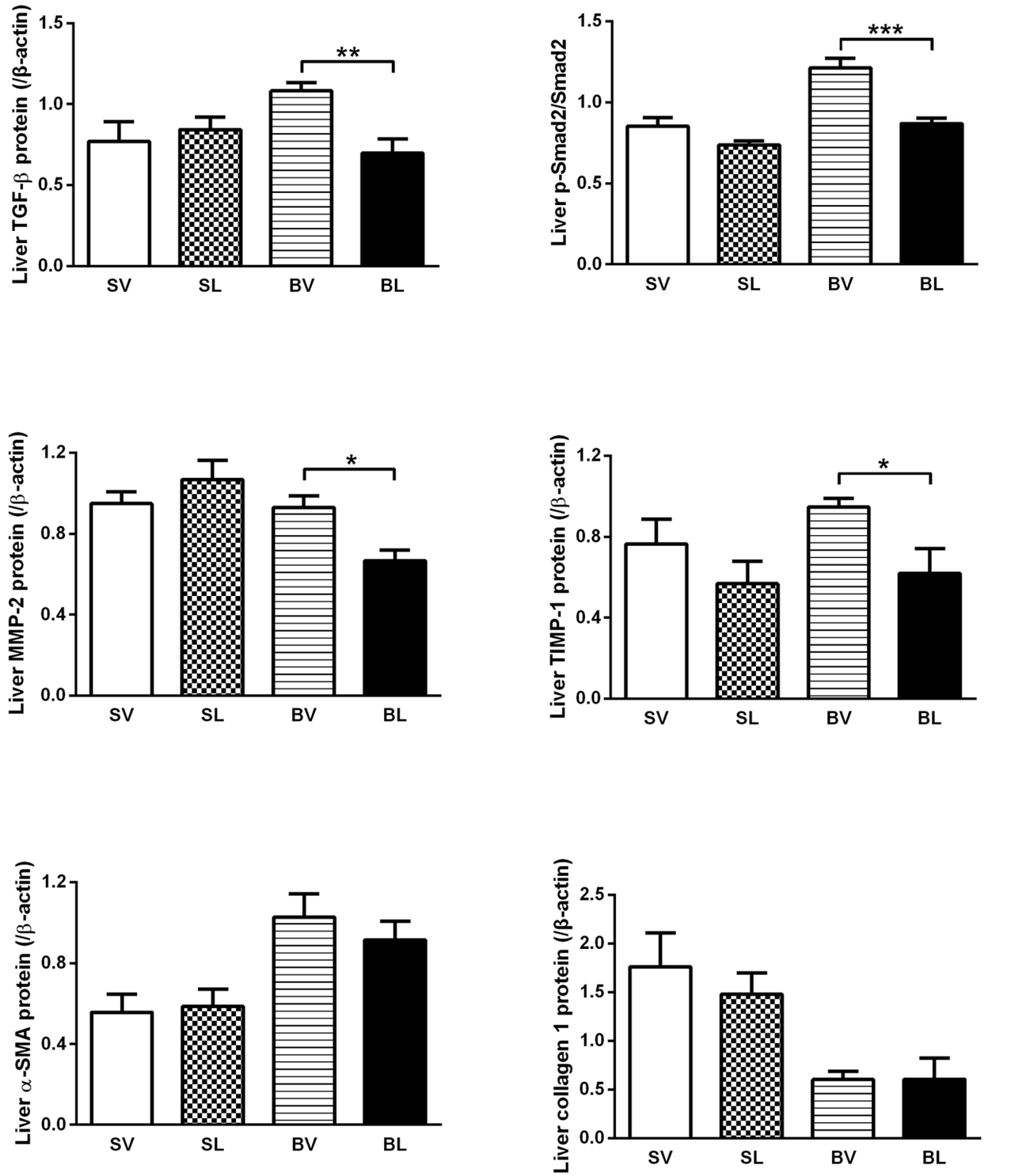


Fig. 5 The hepatic fibrogenic protein expressions in sham and BDL rats with vehicle or levocetirizine administration. As compared with the vehicle, levocetirizine significantly down-regulated TGF-β, p-Smad2, MMP-2, and TIMP-1 expressions ($p < 0.05$). BDL = bile duct ligation.

levocetirizine also reduced SMA blood flow, which contributed to its portal hypotensive effect. Previous studies have shown that histamine-induced endothelium-dependent relaxation by stimulation of H1 receptors in the mesenteric arterial bed.[7] This is compatible with our finding that H1 receptor antagonism by levocetirizine could inhibit histamine-induced mesenteric arterial vasodilatation, followed by reduced SMA blood flow and portal pressure.

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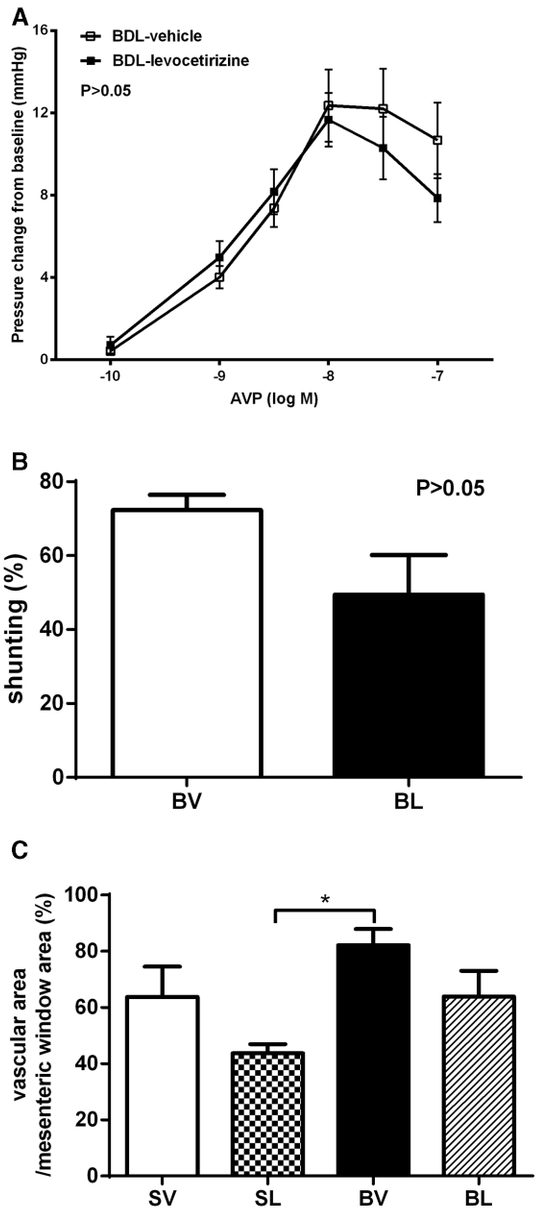


Fig. 6 A, The portosystemic collateral vascular bed concentration-response relationship to arginine vasopressin in BDL rats treated with vehicle or levocetirizine. There were no significant changes in perfusion pressures between groups in response to different concentrations of arginine vasopressin ($p > 0.05$). B, The portosystemic shunting degree in BDL rats treated with vehicle or levocetirizine. Although levocetirizine tended to reduce the shunting degree in BDL rats, the statistical significance was not reached ($p > 0.05$). C, The mesenteric window vascular area in BDL rats treated with vehicle or levocetirizine for 28 days. Levocetirizine significantly reduced the vascular density in sham rats. Levocetirizine tended to reduce the shunting degree in BDL rats; the statistical significance was not reached ($p > 0.05$). BDL = bile duct ligation.

TGF-β is a central regulator in chronic liver disease, contributing to fibrogenesis[26] and Smad-2 and Smad-3 are two major downstream regulators that promote TGF-β1-mediated tissue fibrosis.[27] Furthermore, gelatinases, including MMP-2, play pivotal roles in the development of liver fibrosis, because they degrade basal membrane collagen and are involved in the early steps of tissue remodeling.[28] In terms of the molecular actions of histamine, histamine-induced lung fibroblast proliferation and connective tissue growth factor (CTGF) expression.[29] CTGF is a downstream factor of TGF-β1 that regulates fibrogenesis and angiogenesis.[30] It has been shown that the histamine-induced CTGF expression was mediated through the H_1 receptor.[31] The previous study has also identified that Smad-3, a member of the Smad family, was stimulated by histamine.[32] MMP-2 could be induced by histamine as well.[33] Consistent with our findings about the anti-fibrosis effect of levocetirizine, a previous survey reported that levocetirizine inhibited rhinovirus-induced up-regulation of fibrogenic and angiogenic factors, including MMP-2, MMP-9, VEGF, and TGF-β in nasal polyp fibroblasts.[34] Therefore, the inhibition of TGF-β, p-Smad2, and MMP-2 by levocetirizine may help reduce the intrahepatic fibrosis and resistance, hence contribute to the reduction of PP.

Another factor that may influence the development and maintenance of portal hypertension is mesenteric angiogenesis. With CD31 immunofluorescence staining of the mesenteric window, we found that levocetirizine tended to reduce the mesenteric vascular area in BDL rats, but statistical significance was not reached. It has been noted that histamine stimulated neovascularization through both H1-and H2-receptors in normal healthy rats.[35] A study using carrageenin-induced air pouch-type inflammation animal model revealed that histamine-induced VEGF protein expression and angiogenesis in the carrageenin-induced granulation tissue,[36] which was inhibited by cimetidine, a H2 receptor blocker. It is possible that the H2 receptor is also involved in histamine-related mesenteric angiogenesis, and that angiogenesis could only be partially inhibited by H1 antagonism in our experimental setting.

The limitation of the current study lies in several aspects: First, antihistamines could induce drowsiness, somnolence, and impaired cognitive function, which might adversely affect cirrhotic patients. Although we have chosen levocetirizine, the one that lacks the CNS effect, cautions still have to be paid. Second, BDL is an animal model with cholestatic liver damage, so the findings may not be applicable to cirrhosis related to other etiologies. Third, although hepatotoxicity of antihistamines is not common,[37] the use of antihistamines in cirrhotic subjects with limited liver reserve should be used with caution. The recommended dose of levocetirizine to treat pruritus for adults is 5 mg per day, approximately 0.083 mg/kg/day in a 60-kg person. According to the dose conversion between animals and humans,[38] the dose for the rat is estimated to be 6.2 times higher than that for humans. Therefore, the safe dose for rats is around 0.51 mg/kg/day, which is similar to what we used in the current study, and the liver biochemistry parameters were not influenced in BDL cirrhotic rats. Overall, although antihistamine seems to be promising in controlling liver cirrhosis and portal hypertension-related derangements in cirrhotic rats without inducing further liver injury, the translational clinical use still requires further investigation.

In conclusion, levocetirizine, a H1-blocker that is frequently used to control pruritus in cirrhotic patients, is capable of ameliorating liver fibrosis, reducing portal pressure, SMA flow, PV flow and increasing PV resistance without influencing portosystemic collateral vascular responsiveness to vasoconstrictor, shunting degree, mesenteric angiogenesis and liver/kidney biochemistry parameters. The beneficial portal hypotensive effect may be attributed to the downregulation of hepatic fibrogenesis factors. The clinical application may require further evaluation.

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ACKNOWLEDGMENTS

This work was supported by National Science and Technology Council (105-2314-B-182A-123) and Taipei Veterans General Hospital (V108C-099). The authors thank Yun-Chien Hsieh and Shu-Yu Chang for their technical assistance.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at https://links.lww.com/JCMA/A354.

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