Effects of hepatic blood inflow on liver ultrastructure and regeneration after extensive liver resection in rats with cirrhosis

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Abstract. The aim of the present study was to investigate the effects of hepatic blood inflow on liver function, liver ultrastructure and the regeneration of future liver remnant (FLR) following major hepatectomy in rats with liver cirrhosis. A rat model of cirrhosis was established through intraperitoneal injection of carbon tetrachloride for 8 consecutive weeks. Extensive liver resection and different blood inflow models by portal vein (PV) and/or hepatic artery (HA) stenosis were conducted on the cirrhosis rats. Animal models were constructed as follows: Control (group A), low-flow PV + high-flow HA (group B), low-flow PV + low-flow HA (group C), high-flow PV + high-flow HA (group D) and high-flow PV + low-flow HA (group E). Hepatic blood inflow was detected by laser speckle contrast analysis, liver function and pathological changes were analyzed, Masson staining was used to identify the fibrosis of the liver and Periodic acid-Schiff staining was used to identify glycogen synthesis and hepatocyte function. The liver cell ultrastructure was evaluated by transmission electron microscopy, and the expression of Ki-67 in hepatocytes and the weight of the FLR were recorded to determine the regeneration of the FLR. Five days after major hepatectomy and liver blood inflow modulation, pathological examination of the livers from groups B and C revealed less congestion and less extensive hepatocellular injury. The serum alanine aminotransferase level of group B at 1, 3 and 5 days after hepatectomy and blood inflow modulation was 460.9±31.7, 331.0±22.0 and 285.6±15.8 U/l, respectively (control group: 676.9±41.7, 574.9±28.0 and 436.1±32.7 U/l, respectively; P<0.05); the total bilirubin of group B at 1, 3 and 5 days was 20.4±1.5, 16.1±1.0 and 13.5±0.6 μmol/l, respectively (control group: 30.3±1.4, 26.5±0.8 and 22.1±1.2 μmol/l, respectively; P<0.05). The size of the endoplasmic reticulum in the low-flow PV groups increased significantly and the mitochondrial swelling was alleviated. The positive rate of Ki-67 in the hepatocytes of groups B, C and D was 23.9±3.6, 15.7±2.3 and 12.9±2.4%, respectively (control group: 10.1±2.1%, P<0.05), and the positive rate of Ki-67 in group E was 6.1±1.4% (compared with that of the control group, P<0.05). The remnant liver weight of group B was 15.4±1.0 g (compared with that of the control group, P<0.05). Therefore, decreased portal blood flow combined with increased hepatic arterial blood flow alleviated the congestion in the liver following major hepatectomy in cirrhotic rats, improved the pathological status and liver function, increased the expression of Ki-67 and promoted liver regeneration.

Introduction

Liver resection remains the treatment of choice for primary liver cancer. However, patients with cirrhosis and portal hypertension may develop portal hyperperfusion (PHP) following major hepatectomy, which may lead to post-hepatectomy liver failure (PHLF) (1-4). In literature, the reported mortality of posthepatectomy liver failure is <5% and morbidity is 15-30%. Around 3-8% of patients develop liver failure after major hepatectomy (5,6). The elevated hepatic sinusoidal pressure caused by PHP may damage hepatocytes and sinusoidal endothelial (SE) cells and eventually impede liver regeneration (3,4). It was hypothesized that artificially decreasing the portal flow following major hepatectomy may alleviate the damage caused by PHP (2,7). The liver receives portal as well as arterial blood, and is modulated by the hepatic arterial buffer response, as the increase of portal blood flow may cause a decrease in hepatic arterial blood flow and vice versa (8,9). With PHP, the hepatic arterial blood flow may decrease, leading to decreased oxygen supply, which may cause further damage to the future liver remnant (FLR). Therefore, the hepatic arterial blood flow may also be modulated to increase the oxygen supply in order to protect and promote the regeneration of the FLR. However, studies on the damage of PHP to the liver and its effects on liver regeneration are based on living donor liver transplantation and defined as 'small-for-size' syndrome, rather than on major hepatectomy for patients with cirrhosis. A considerable proportion of primary liver cancer patients have concurrent cirrhosis and portal hypertension; furthermore, hepatic hemodynamics is complex and major hepatectomy may further complicate this condition. In addition, there are

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more cases of major hepatectomy compared with living donor liver transplantation. In order to mimic the clinical conditions, a rat model of cirrhosis was established in this study, followed by major hepatectomy and stenosis of the portal vein (PV) and/or hepatic artery (HA) to establish different hepatic inflow volumes, and to evaluate the effect of hepatic inflow on liver ultrastructure, function and regeneration.

Materials and methods

Reagents and instruments

Experimental reagents. Anhydrous ethanol, xylene, hydrochloric acid, embedding paraffin, neutral gum, 2.5% glutaraldehyde fixative, Masson staining reagents, and other related chemical reagents were purchased from Sinopharm Group (Shanghai, China); PAS staining kit was purchased from Wuhan Servicebio Company (Wuhan, China). CCL_4 was purchased from Suzhou Second Chemical Research Institute (Suzhou, China); hematoxylin was purchased from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany).

Experimental instruments. The RM 2016 Microtome was obtained from Leica Microsystems GmbH (Wetzlar, Germany); the automatic biochemical analyzer was purchased from Beckman Coulter, Inc., (Brea, CA, USA); the CX31 Upright Microscope was purchased from Olympus Corporation, (Tokyo, Japan); the Laser Speckle Imager was from Perimed AB (Järfälla, Sweden); and the transmission electron microscope was obtained from JEOL, Ltd., (Tokyo, Japan).

Experimental protocol

Establishment of rat model of cirrhosis. A total of 50 male Sprague-Dawley rats, aged ~8 weeks and weighing 300-320 g, were purchased from Zhaoyan New Drug Research Center Co., Ltd., (Suzhou, China) (animal quality certificate: SCXK 2013-0003). The animals were treated humanely following the Institutional Guidelines for Animal Use and Care at the Institute of Zoology, the Chinese Academy of Sciences. The present study also complied with the management rules of the National Health and Family Planning Commission of China and was approved by the Ethics Committee of Zhejiang Cancer Hospital. All animals were kept clean and in sanitary conditions, with the room temperature maintained at 20±2˚C, relative humidity at 65±10%, in a diurnal cycle. The animals were randomly assigned to CCL_4-treated (model, n=43) and non-CCL_4-treated (n=7) groups. The process of establishment of the cirrhosis model was divided into induction and modeling stages. During the induction stage, the rats were fed a normal diet and a solution of 0.35 g phenobarbital sodium per liter of distilled water was administered as the only drinking water for 1 week. During the modeling stage, 40% CCL_4 neutral rapeseed oil solution was injected intraperitoneally (i.p.) at a dose of 0.5 ml/100 g twice a week for 4 consecutive weeks; 10% ethanol solution was given as the only drinking water. From the fifth week, 50% CCL_4 neutral rapeseed oil solution was injected i.p. at a dose of 0.5 ml/100 g at the same frequency for 4 consecutive weeks; 30% ethanol solution was given as the only drinking water until the end of the eighth week. In the non-CCL_4-treated group, saline at a dose of 0.3 ml/100 g body weight was injected i.p. twice weekly, and the animals were given ordinary drinking water and food.

Liver resection + hepatic inflow control during the first laparotomy. Rats with liver cirrhosis were fasted for 12 h with access to water *ad libitum*. After being anesthetized with i.p. injection of 0.3% sodium pentobarbital (40 mg/kg), the rats underwent laparotomy by a middle incision in the upper abdomen. The liver's texture, PV system and possible presence of ascites were evaluated; the left lateral lobe and left medial lobe of the liver (accounting for ~45% of the total liver volume) were removed (10). The liver specimens of the non-CCL_4-treated group were collected for pathological analysis. After liver resection, different hepatic blood inflow was established for the CCL_4-treated group and these animals were assigned to different groups (n=7 per group) as follows:

Group A: Control group, no blood inflow intervention.

Group B: Low-flow PV + high-flow HA group (the PV was narrowed and the splenic artery was ligated).

Group C: Low-flow PV + low-flow HA group (the PV and the HA were narrowed).

Group D: High-flow PV + high-flow HA group (the splenic artery was ligated).

Group E: High-flow PV + low-flow HA groups (the HA was narrowed).

The procedure undertaken to narrow the PV was as follows: The PV was exposed, a no. 8 needle was placed in parallel with the main PV trunk, the PV was double-ligated and then the needle was withdrawn, ensuring that the diameter of the narrowed PV was approximately equal to that of the needle. Intrahepatic blood flow was measured by laser speckle contrast analysis (LASCA) to ensure a 30% decrease compared to the data after major hepatectomy. To narrow the HA, an insulin needle was used, with the same method applied as for narrowing the PV. To establish high flow to the HA, the splenic artery was ligated.

Liver function tests. Serum was collected before and after establishment of the cirrhosis model, and at 1, 3 and 5 days after hepatic inflow regulation and liver resection, and was analyzed by an automatic biochemical analyzer.

Measurement of the liver blood flow with LASCA. During the first surgery, the data of liver blood flow were collected at three different time points: Before hepatectomy, after hepatectomy and after hepatectomy + blood flow regulation. At 5 days after the first surgery, the second laparotomy was performed and the data of liver blood flow were again collected. Of note, due to the need to excise the left lateral and left medial lobes of the liver, the LASCA probe must be aimed at the right lateral and right medial lobes of the liver (FLR) to measure the blood flow changes throughout the experiment (11).

Weight of FLR. After the liver blood flow measurement in the second laparotomy, the animals were sacrificed humanely and the whole liver was harvested and weighed after removal of excessive fluid and surrounding tissues.

Pathological examination. The liver specimens were collected at different time points and treated as follows: Liver tissue was placed in 10% formaldehyde for 48 h, rinsed with water, and dehydrated using different concentrations of ethanol, followed by embedding in paraffin, sectioning, heating and dewaxing.

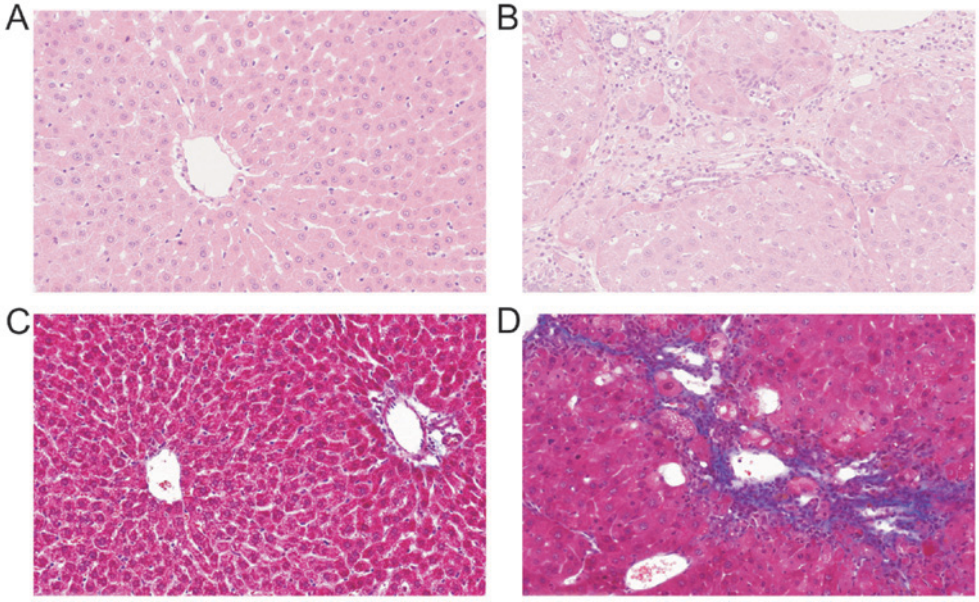

Figure 1. Liver pathological examination verifying the establishment of rat cirrhosis model (A and B: Hematoxylin and eosin; magnification, x200). (A) non-CCL_4-treated group, normal structure; (B) CCL_4-treated group (modeling group); the normal structure was damaged with formation of false flocculus (C and D: Masson staining; magnification, x200). (C) non-CCL_4-treated group, normal structure. (D) The formation of false flocculus and deposition of collagen in the portal area.

The sections were stained with hematoxylin and eosin (H&E) for morphological evaluation and Masson trichrome (MT) to assess the degree of fibrosis. PAS staining was performed following the manufacturer's protocol and the sample was collected and examined.

Evaluation of liver cell ultrastructure. The samples were fixed in 2.5% glutaraldehyde, washed and postfixed in 1% osmium tetroxide, followed by dehydration in a series of ethanols and propylene oxide. The tissues were then embedded in an epoxy resin. Ultrathin sections were cut and mounted on a grid, and the grid was stained with uranyl acetate and lead acetate and observed under a TEM.

Ki-67 expression analysis. Liver tissue was collected and fixed in formaldehyde, washed and dehydrated using graded ethanols, embedded in paraffin, sectioned, and subjected to antigen retrieval and endogenous peroxidase activity inhibition. The primary antibody was prepared with a dilution ratio of 1:100. Each section was incubated with the primary antibody at 4˚C, rinsed 3 times with phosphate-buffered saline (PBS) for 5 min each time, followed by the addition of secondary antibody working fluid and incubation at room temperature in a wet box for 10-15 min. The secondary antibody was then discarded, and the sections were rinsed 3 times with PBS for 5 min each time; each section was placed in the wet box with streptomycin-avidin peroxidase solution at room temperature for 10-15 min, and then the working fluid was discarded and the sections were rinsed with PBS 3 times for 5 min each time, followed by DAB dye staining, hematoxylin re-staining, alcohol dehydration, xylene transparentization and other steps. The final sample was observed under the microscope.

Statistical analysis. SPSS v.19.0 statistical software (SPSS Inc., Chicago, IL, USA) was used for data analysis. All data are expressed as mean ± standard deviation (SD). One-way analysis of variance followed by the Fisher's least significant difference test was used to compare two different groups. $P<0.05$ was considered to indicate statistically significant differences which were plotted with GraphPad Prism v.6 software (GraphPad, Software, Inc., La Jolla, CA, USA).

Results

General observations. In the CCL_4-treated group, 3 rats died during the modeling process due to acute liver injury and liver failure. Compared with the non-CCL_4-treated group, all animals in the CCL_4-treated group were in low spirits and exhibited a lower rate of body weight gain (data not shown). A total of 5 rats died following major hepatectomy due to bleeding (n=3) and PHLF (n=2). The livers of the CCL_4-treated group developed moderate cirrhosis, with formation of a few portosystemic collateral vessels; no obvious ascites was observed.

Pathological identification of cirrhosis. As shown in Fig. 1, the pathological examination revealed disrupted liver architecture, with collagen deposition, diffuse fibrosis and formation of false lobules after the establishment of the model.

Hepatic hemodynamics. There were no significant differences in the intrahepatic blood flow among groups prior to liver resection. After major hepatectomy, the blood flow of the remnant liver in all groups increased significantly (compared with that prior to hepatectomy, $P<0.05$). After major liver resection + hepatic inflow regulation, the intrahepatic blood flow in groups B and C was significantly reduced (compared with groups A, D and E, $P<0.05$). The intrahepatic blood flow in groups D and E was increased to a different degree (compared with groups A, B and C, $P<0.05$). At 5 days after liver resection + hepatic inflow regulation, the aforementioned

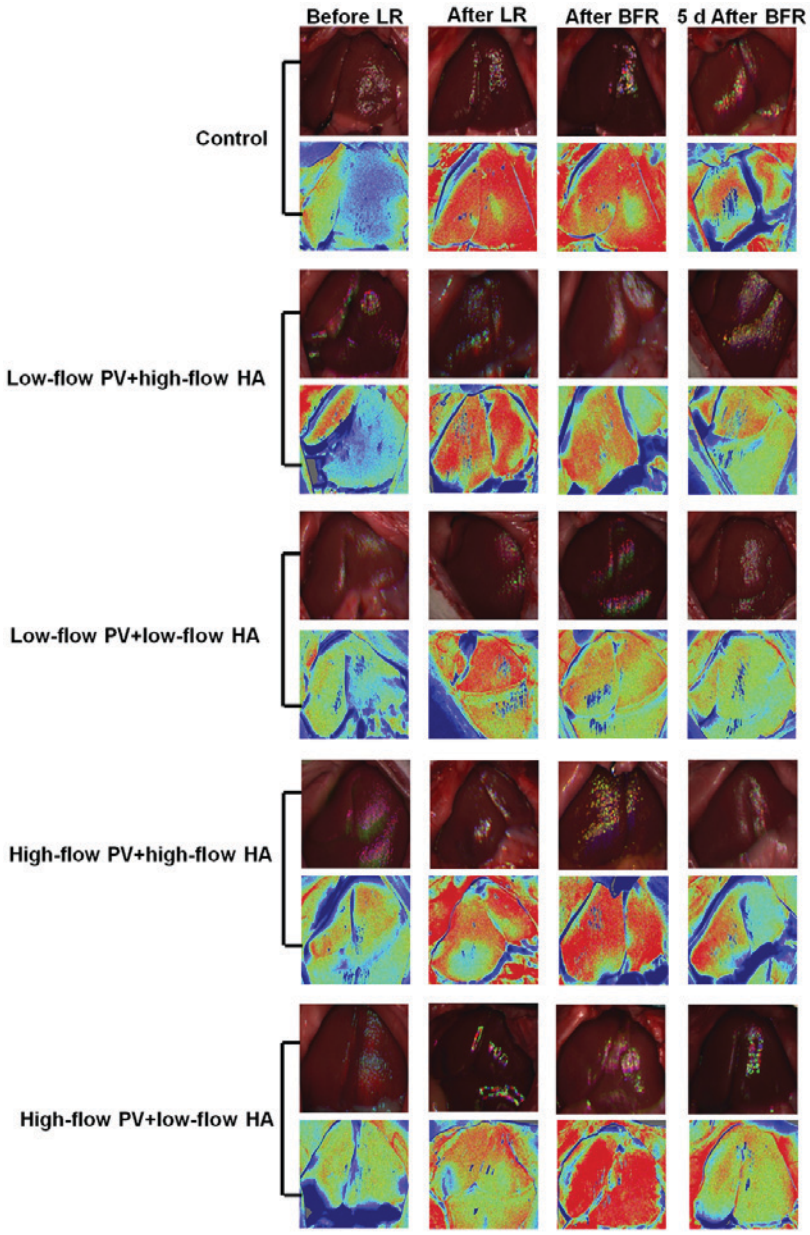

Figure 2. Hepatic inflow detected by LASCA; after hepatectomy and blood flow regulation, the blood inflow changed accordingly: The darker the red part in the image, the higher the blood flow in the region. LR, liver resection; BFR, blood flow regulation.

changes remained, but the differences were less prominent (Figs. 2 and 3).

Liver function. The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL) of CCL_4-treated group increased gradually during the modeling process (Table I). These liver function index of each group increased significantly after hepatectomy + hepatic inflow regulation. The postoperative serum levels of ALT, AST and TBIL in groups B and C were significantly lower compared with those in the control group and groups D and E ($P<0.05$; Table II). The albumin levels remained stable throughout the experiment.

Weight of liver remnant. On postoperative day 5, after the hepatic blood flow examination, the rats were sacrificed and the residual liver was collected for weighing. The weight of the

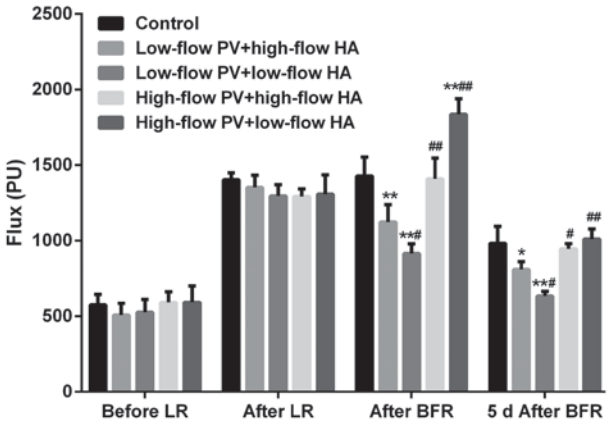

Figure 3. Comparison of the hepatic inflow between each group (vs. Control, $^{*}P<0.05$; $^{**}P<0.01$; vs. Low-flow PV + high-flow HA, $^{\#}P<0.01$, $^{\#\#}P<0.01$). LR, liver resection; BFR, blood flow regulation; Flux, blood flux; PU, flux entering the tissue in unit time; HA, hepatic artery.

Table I. Liver function changes during the establishment of rat cirrhosis model (n=7, each group).

	ALT (U/l)			AST (U/l)			ALB (g/l)			TBiL (μmol/l)		
	0 week	8 week	10 week	0 week	8 week	10 week	0 week	8 week	10 week	0 week	8 week	10 week
Non-CCL_4-treated	38.9±2.1	37.7±2.1	35.7±7.3	116.4±6.0	113.3±8.7	115.6±7.3	30.2±1.3	29.4±1.6	30.1±2.4	0.59±0.2	0.7±0.2	0.7±0.3
CCL_4-treated	38.3±2.6	132.1±7.5	245.3±22.8	116.9±8.2	327.0±11.8	1056.3±109.6	29.9±0.8	30.4±1.9	29.4±1.8	0.53±0.2	6.1±1.2	22.0±4.8
P-value	=0.66	<0.01	<0.01	=0.91	<0.01	<0.01	=0.61	=0.29	=0.53	=0.55	<0.01	<0.01

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin.

Table II. Liver function changes following major hepatectomy and hepatic blood inflow regulation (n=7, each group).

	ALT (U/l)			AST (U/l)			ALB (g/l)			TBiL (μmol/l)		
Group	1 day	3 day	5 day	1 day	3 day	5 day	1 day	3 day	5 day	1 day	3 day	5 day
A	676.9±41.7	574.9±28.0	436.1±32.7	1141±83	941±28	789±30	28.6±2.4	27.6±2.2	27.1±1.9	30.3±1.4	26.5±0.8	22.1±1.2
B	460.9±31.7	331.0±22.0	285.6±15.8	791±27	662±33	552±24	27.7±1.1	29.0±1.6	28.1±2.6	20.4±1.5	16.1±1.0	13.5±0.6
C	551.0±33.0	429.9±28.5	359.1±22.9	937±56	809±36	648±38	28.3±1.6	28.41.9±	29.0±1.6	22.9±1.5	22.6±0.8	17.9±1.3
D	674.7±27.6	588.9±22.6	454.0±18.5	1156±61	937±27	803±28	29.1±2.4	27.8±1.8	28.9±2.3	29.6±1.6	27.5±0.9	23.3±0.9
E	857.0±26.8	727.6±29.8	579.1±38.2	1438±73	1175±59	931±44	29.1±1.1	29.2±2.6	28.8±1.2	38.0±1.7	32.8±1.7	28.8±1.7
P-value[a]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	>0.05	>0.05	>0.05	<0.05	<0.001	<0.001
P-value[b]	0.925	0.379	0.193	0.740	0.603	0.398	0.720	0.831	0.141	0.358	0.101	0.050

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin. [a]Compared between each group except the comparison between group A and group D. [b]Comparison between group A and group D.

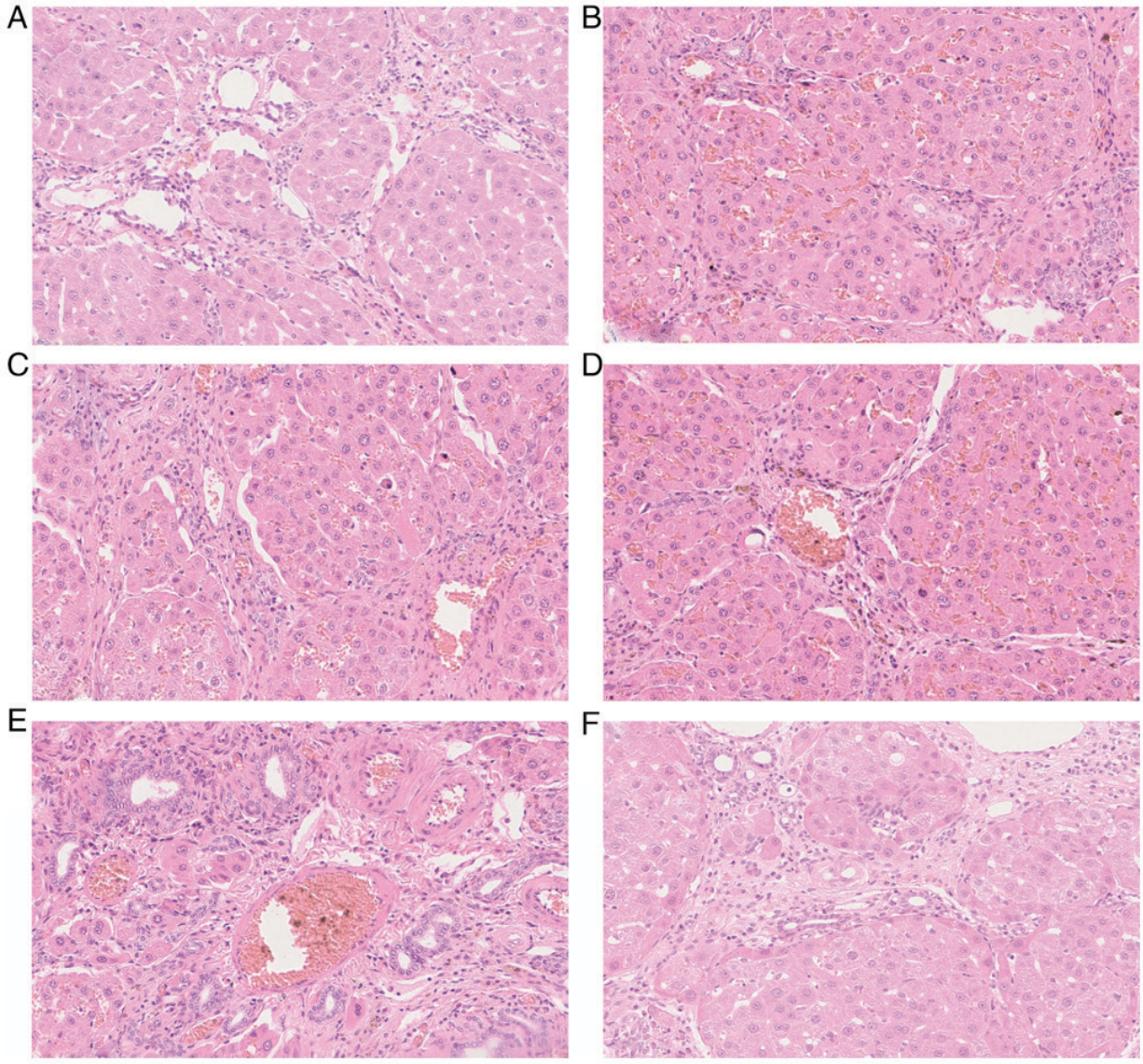

Figure 4. Pathological examination of remnant liver tissue (hematoxylin and eosin staining; magnification, x200). The results revealed the congestion was improved in the low-flow PV groups (B and C). (A) Control group; (B) group B; (C) group C; (D) group D; (E) group E; (F) resected liver tissue in the first lapalotomy.

FLR was: Group A: 11.8±0.7 g, group B: 15.4±1.0 g, group C: 13.1±1.2 g, group D: 11.8±0.6 g, group E: 10.9±1.0 g, respectively. The results demonstrated that the residual liver weight in the low-flow PV groups was significantly higher compared with that in the control group and the high-flow PV groups ($P<0.05$).

Liver pathological examination and ultrastructure examination. Routine HE staining of the remnant liver tissues revealed the congestion was improved in the low-flow PV groups (B and C), although it was still obvious in group B, and the hepatic sinus structure and liver cell damage were significantly improved compared with the high-flow PV groups (Fig. 4). The PAS staining of the remnant liver tissues revealed that the accumulation of glycogen was increased significantly in group B and group C (Fig. 5) In the high-flow PV groups, the mitochondrial swelling of the hepatocytes was more prominent, the mitochondrial ridge structure was loose and dissolved, the membrane structure was blurred, and the endoplasmic reticulum was significantly decreased (Fig. 6). There was more endoplasmic reticulum in the low-flow PV groups, the mitochondrial swelling was alleviated (it was more obvious in group C compared with that in group B), and the mitochondrial ridge was clear.

Ki-67 expression. There was no significant difference in Ki-67 expression among groups prior to liver resection + blood inflow regulation. The Ki-67 expression in groups B and C was significantly increased, particularly in group B (compared with groups A, D and E, $P<0.05$). The expression of Ki-67 in group D was also increased compared with group A, but was lower compared with that in groups B and C. The Ki-67 expression in group E was significantly lower compared with that in the control group ($P<0.05$) (Figs. 7 and 8).

Discussion

In human, resection of three or more segments, or ≥4 liver segments was defined as major hepatectomy. In the present

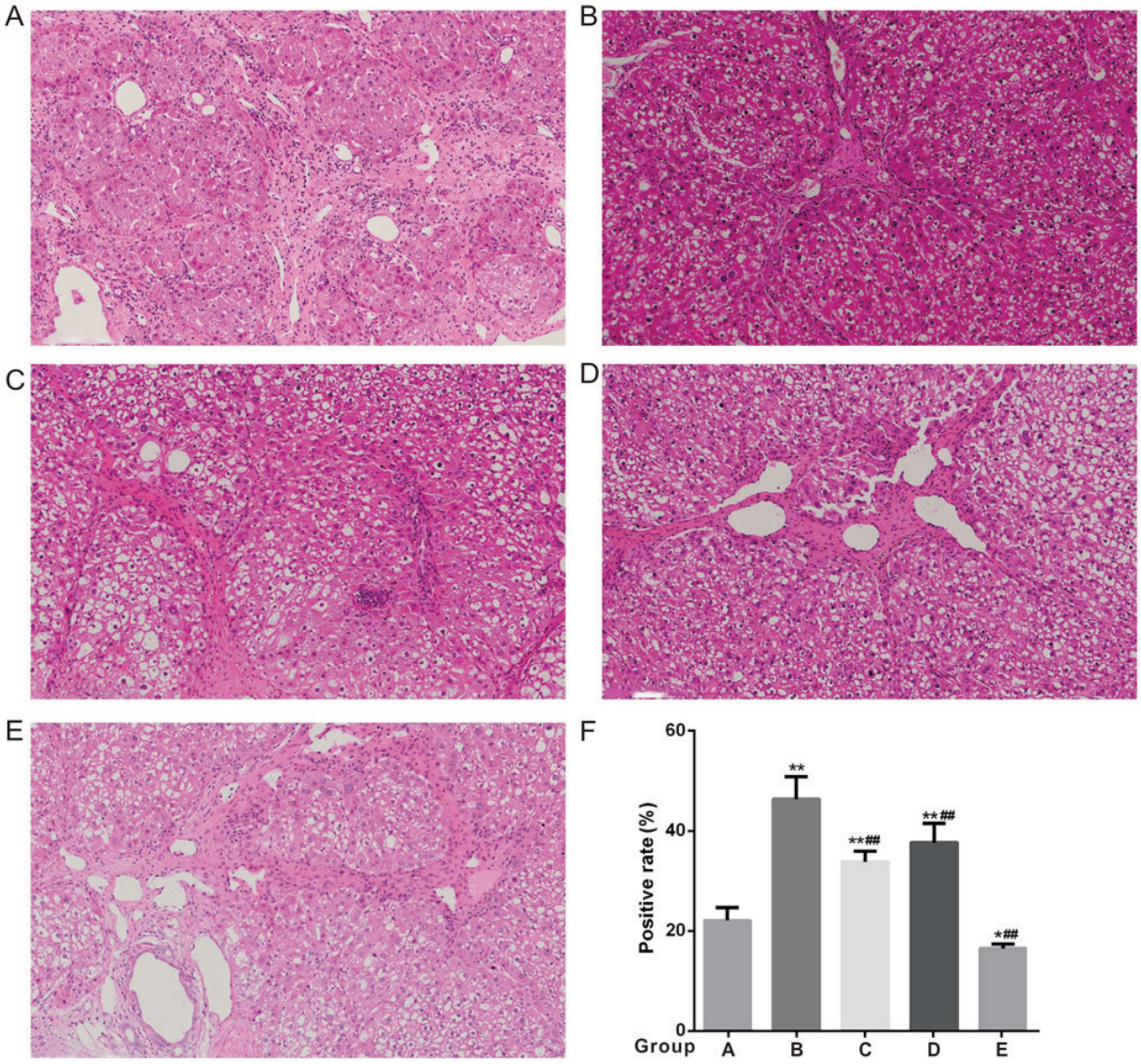

Figure 5. Evaluation of the function of hepatocyte glycogen synthesis by PAS staining (magnification, x100). The accumulation of glycogen was increased significantly in group B and group C. (A) Control group; (B) group B; (C) group C; (D) group D; (E) group E; (F) comparison of the positive rate between each group (vs. Control, *P<0.05; **P<0.01; vs. Low-flow PV + high-flow HA, $^{\#\#}$P<0.01). HA, hepatic artery.

study, the removal of the left lateral lobe and left medial lobe of the liver (accounting for ~45% of the total liver volume) was defined as major hepatectomy (10,12). Following major hepatectomy, the cross-section of the intrahepatic vascular bed is significantly reduced and the residual liver receives the blood that formerly flowed through the whole liver, resulting in PHP (13,14). Although a slightly increased portal pressure on liver SE cells may promote liver regeneration (2), the damage caused by excessive pressure and excessive flow may prevail. PHP may cause SE cells swelling and detachment by increased shear stress, impede oxygen diffusion and oxygen uptake by the liver tissue (2,3,15). Liver regeneration requires a rich supply of oxygen and energy (16,17), while PHP reduces the oxygen saturation in the liver tissue, limiting the oxygenation of liver cells and reducing the mitochondrial redox function, Dold *et al* revealed that the injury to mitochondria by PHP following major hepatectomy may be related to oxygen supply and oxygen uptake (13). In that study, hepatic tissue oxygen pressure (pO_2) was significantly decreased and was associated with an increased expression of hypoxia-inducible factor-1α (HIF-1α) under conditions of PHP after a 70 and 90% hepatectomy; in line with these findings, 90% hepatectomy also resulted in an increase of NADH fluorescence compared with the baseline, indicating an impaired mitochondrial redox status of the liver tissue. Mitochondria are the only energy-supplying organelles in the cells; thus, mitochondrial damage may cause disordered intracellular metabolism and lead to cell necrosis and apoptosis. Following hepatectomy, mitochondrial DNA replication and translation increase, and mitochondrial respiratory function is significantly increased. These activities are closely associated with liver regeneration (18–21). Moreover, it is hypothesized that mitochondria may be a potential regulatory site to initiate hepatocytes entering the division cycle (18). In the present study, pathological examination revealed that the congestion was alleviated in the low portal flow groups; in addition, the disruption in sinusoidal structure and hepatocyte injury were significantly attenuated. The ultrastructural study

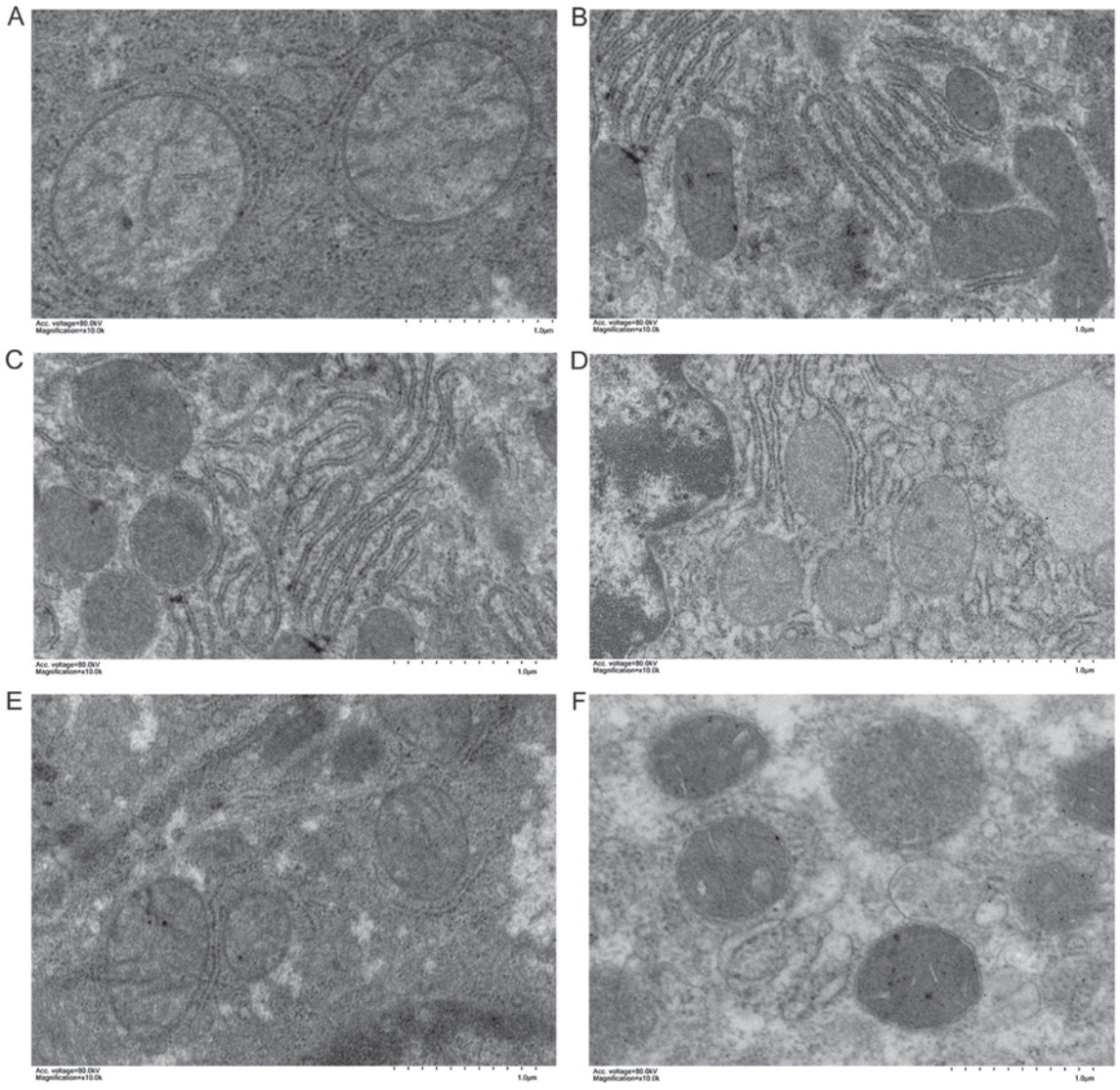

Figure 6. Ultrastructure of the hepatocyte by transmission electron microscopy (magnification, x7,000). (A-E) Remnant liver tissues 5 days after liver resection and blood flow regulation. (B and C) The structure of mitochondria was more intact in the low-flow PV groups. (A) Control group; (B) group B; (C) group C; (D) group D; (E) group E; (F) resected liver tissue in the first lapalotomy.

by TEM revealed that the damage to the mitochondria was more severe in the high portal flow groups compared with that in the low portal flow groups. These results show that correcting the state of PHP and alleviating the damage of SE cells by PHP may improve the oxygen supply of liver tissue and the consumption of oxygen by liver tissue, thereby reducing the damage to mitochondria. It is the limitation of the present study not to evaluate the function of mitochondria. In the future study, we will examine at least two of the respiratory complex including Complex I, Complex II, Complex III or Complex IV in order to analyze the active status of the mitochondria. It's also a limitation of the study that not perform the Masson staining through the whole experiment process. In the future study, we will perform Masson staining to monitor the changes of the degree of fibrosis to evaluate the possible impact of hepatic blood inflow on the liver fibrosis.

The regeneration of the FLR is the basis for the recovery of liver function following major hepatectomy. Liver regeneration is triggered 0-4 h after liver resection or acute injury. There are three main stages of hepatocyte regeneration: The initiation phase (G0-G1 phase), the proliferation phase (G1-S phase) and the termination phase (G1-G0 phase) (22,23). Ki-67 is located in the nucleus and is a marker of cell proliferation; it can mark most cells, except those in the G0 phase. A high positive rate of Ki-67 in hepatocytes indicates a higher proportion of cells in the proliferative cycle and faster regeneration (24). As PHP has a negative effect on the regeneration of the FLR, correcting PHP may promote liver regeneration. A series of measures to decrease portal pressure have been adopted in clinical settings to alleviate damage and provide a more favorable physiological environment for the regeneration of FLR (13,25,26). For example, Bucur *et al* (14) promoted the regeneration of the FLR and reduced the incidence of PHLF by restricting portal flow with a ring-shaped device after major hepatectomy in pigs. As the liver receives a dual blood supply (PV and HA) and is regulated by the HA buffer response, the PHP after major hepatectomy may affect HA blood flow and have an additional significant impact. By improving the HA blood flow, liver regeneration and liver function may improve. Eipel *et al* (27) reported that removal of ~85% of the liver in rats resulted in a 40% increase in residual

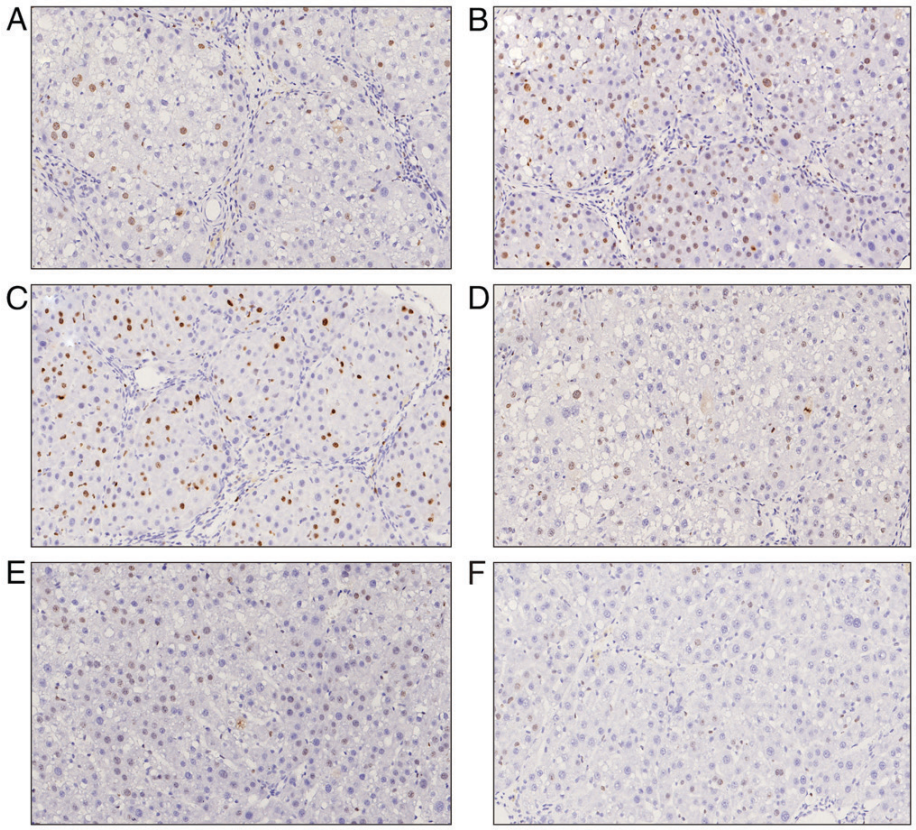

Figure 7. Immunohistochemistry evaluation of Ki-67 expression (magnification, x200). (A-E) Remnant liver tissues 5 days after liver resection and blood flow regulation. (B and C) The construction was more intact in the low-flow PV groups. (A) Control group; (B) group B; (C) group C; (D) group D; (E) group E; (F) resected liver tissue in the first lapalotomy.

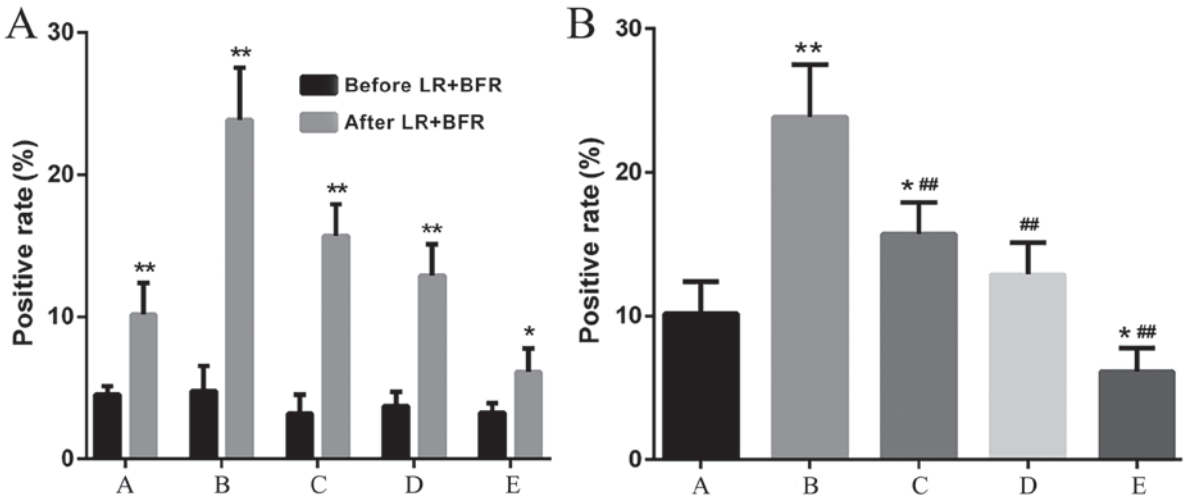

Figure 8. Comparison of Ki-67 expression between each group. (A) Comparison of each group before and 5 days after liver resection (LR) + blood flow regulation (BFR) (*P<0.05; **P<0.01); (B) comparison between each group 5 days after LR+BFR (vs. Control, *P<0.05; **P<0.01; vs. Low-flow PV+high-flow HA, $^{\#\#}$P<0.01). A-E under abscissa: (A) control group; (B) group B; (C) group C; (D) group D; (E) group E. HA, hepatic artery.

hepatic portal flow, a 50% decrease in liver tissue oxygen uptake and a significant increase in the mortality rate in rats; when the HA blood supply was increased by spleen resection, the prognosis of rats also improved. In the present study, the expression of Ki-67 in the low portal flow groups, particularly in the low portal flow + high arterial flow group, was significantly increased and the weight of FLR was significantly increased. The regeneration of the FLR was consistent with the pathological changes following major hepatectomy, and the blood inflow regulation may play an important role through the protection of the liver structure: Limiting the injury of liver tissue is associated with higher oxygen supply, normal structure and function of the mitochondria, and more efficient liver regeneration. Following major hepatectomy, the epitelial bili, endotelial cells, Küpffer, as well as hepatocytes begin to regenerate rapidly. We will detect the hepatocyte markers including alfa-fetoprotein, hexokinase

or piruvate kinase in the future study to determine whether regeneration is primarily from hepatocytes or other cells.The recovery of function of the FLR is based on the intact structure and rapid regeneration of the FLR. The present study demonstrated that the levels of serum ALT and TBIL of the low portal flow groups were closest to normal, PAS staining of the liver tissue showed that the glycogen synthesis by hepatocytes of the low portal flow groups was better than other groups, indicating that the decrease of the portal flow may significantly improve the function of the FLR following major hepatectomy. With the improvement of PHP, the increase of HA blood flow may have a beneficial effect on the recovery of liver function, likely due to the increased oxygen supply. However, on the basis of PHP, increasing the HA flow cannot effectively alleviate the tissue damage and improve liver function, suggesting that the injury caused by PHP exceeds the improvement associated with the increase of HA flow.

In the present study, the research was based on a cirrhosis rat model, which was different from the majority of previous studies. A considerable proportion of hepatectomies have to be performed on patients with chronic hepatitis and cirrhosis, which are often concomitant with portal hypertension. However, further research is required to elucidate the mechanism underlying the damage resulting from PHP after major hepatectomy. First, as physical damage caused by PHP is a possible explanation, measures should be taken to adequately reduce the pressure on SE cells and hepatocytes; second, oxygen supply to the liver tissue and oxygen consumption by liver tissue should be a focus of further research; finally, as the key organelles of cellular oxygen metabolism, the structure and function of mitochondria should also be investigated. Clinically, preoperative liver blood flow should be evaluated, especially for patients with cirrhosis. Intraoperatively, portal flow as well as hepatic arterial flow should be assessed again after major hepatectomy. If PHP exists, it is necessary to narrow the portal vein properly to reduce the portal pressure and portal flow to avoid damage to the liver. The spleen artery could be appropriately narrowed to increase the hepatic arterial blood flow in order to bring more oxygen to the liver. Further research may help find strategies for protecting and promoting the regeneration of the FLR, in order to ensure safe recovery following major hepatectomy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WJ, JG, XW and YZ were responsible for the design and implementation of experiments; SF and BY were responsible for the management of animals, the collection of specimens and pathological examinations; RD and BW were responsible for the data collection and analysis, and the production of figures. WJ, YZ, JG and XW were responsible for writing of the manuscript.

Ethical approval and consent to participate

The animals were treated humanely following the Institutional Guidelines for Animal Use and Care at the Institute of Zoology, the Chinese Academy of Sciences. The study also complied with the management rules of the National Health and Family Planning Commission of China and was approved by the Ethics Committee of Zhejiang Cancer Hospital.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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