

Original Article

Improved donor liver position selection and revascularization for heterotopic auxiliary liver transplantation with portal vein arterialization

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Abstract: Purpose: To establish an animal model of improved donor liver position selection and revascularization for heterotopic auxiliary liver transplantation with portal vein arterialization (HALT-PVA). Methods: Sprague-Dawley rats were utilized to establish models. Improved HALT-PVA was conducted for the experimental rat: hepatic common artery of donor liver was end-to-side anastomosed to portal vein which was end-to-side anastomosed to the left common iliac artery of host rat, while the segments of inferior vena cava superior and inferior to the donor liver were end-to-side anastomosed to the inferior vena cava of host rat, respectively. For the control rats, liver transplantations were conducted through end-to-end anastomosis between portal vein of donor liver and stand tube placed in right renal artery of host rat, and end-to-side anastomosis between the inferior vena cava inferior to the donor liver with the inferior vena cava of host rat, while the inferior vena cava superior to the donor liver was stitched up. Besides, hepaticoenterostomy were performed to all rats and survival status were monitored. ALT, AST, TBil and CHE were tested continuously after operation, and pathological examination of liver tissues were performed. Results: The survival rate was 93.3% (14/15). ALT, AST, TBil and CHE for experimental group showed a rapider recovery of liver functions than controls. Pathological examinations of liver tissues from the experimental-group rats showed better presentation than the control-group rats. Conclusions: The improved HALT-PVA better accords with the normal anatomy, with little detriment to implanted liver, and therefore is a good model for HALT-PVA related research.

Keywords: Heterotopic auxiliary liver transplantation, portal vein arterialization, revascularization, liver function

Introduction

Portal vein arterialization (PVA) refers to building up blood path between artery and vena portae or one of its branches, in order to realize liver perfusion via increasing arterial blood or replacing blood from vena portae [5, 7, 8]. PVA is mainly applied in liver transplantation when blood flow of portal vein is unavailable to restore [1]. However, the clinical outcomes of orthotopic liver transplantation with PVA have been controversial [1, 3, 16]. Heterotopic auxiliary liver transplantation with portal vein arterialization was first described and recommended by Erhard et al for its advantage over the orthotopic auxiliary liver transplantation in avoiding portal vein thrombosis [3]. Although PVA have

been reported in several successful cases of liver transplantation in recent years [1, 3], it is still a salvage procedure in nature to restore liver blood perfusion, considering the alteration of normal anatomy of liver blood supply. Thus, PVA is still under debate, mainly because that the alteration in blood constituents, blood volume, blood flow rate and blood pressure of portal vein may cause acute reperfusion injury to the donor liver, which is harmful to regeneration.

In this study, an improved heterotopic auxiliary liver transplantation with PVA (IHALT-PVA) will be verified on Sprague-Dawley (SD) rats via optimizing placement and vascular reconstruction of the donor liver, aiming to decrease the

blood volume, blood flow rate and blood pressure of portal vein, and thereby facilitating the regeneration of donor liver. We assumed that IHALT-PVA may be an ideal operational modal for studying PVA after liver transplantation.

Materials and methods

Animals and experiments

Sixty SPF male Sprague-Dawley (SD) rats with weights ranging from 210 to 260 grams were purchased (from Laboratory Animal Center, The Third Military Medical University, Chongqing, China) and classified evenly into the experimental group and the control group. Liver transplantations for rats from the experimental group were performed as follows: hepatic common artery of donor liver was end-to-side anastomosed to its portal vein which was then end-to-side anastomosed to the left common iliac artery of the host rat to establish PAV, while both the segments of inferior vena cava superior and inferior to the donor liver were end-to-side anastomosed to the inferior vena cava of the host rat, respectively. In contrast, liver transplantations for rats from the control group were conducted through end-to-end anastomosis between portal vein of donor liver and stand tube placed in right renal artery of the host rat beforehand, and end-to-side anastomosis between the inferior vena cava inferior to the donor liver with the inferior vena cava of the host rat, while the inferior vena cava superior to the donor liver was stitched up. Besides, hepaticoenterostomy were performed to all rats from both groups. Both donor and recipient rats were fasted at least 12 h before operation, without deprivation of drinking. All processes referring to animal breeding and operation comply with the Regulations for the Administration of Affairs Concerning Experimental Animals, promulgated by the State Science and Technology Commission of People's Republic of China.

IHALT-PVA model establishment

All liver transplantations were performed under 4× to 6× microscopic field, with heparin saline keeping infusing into the abdominal aorta at a rate of 150 ml/h and at a temperature of 4°C. Rats were anesthetized during all procedures by continuously inspiration of isoflurane, while inspiring rate was adjusted appropriately

according to the change of the rats' breathing frequencies and depth, as well as the change of their liver colors.

PVA model for rats from experimental group

For donor liver excision, anesthetic rat was placed on the operation table in supine position, with four limbs and head fixed, and waist pillowed a bit up so as to better expose the liver. Then the rat was heparinized by injection 0.3 ml heparin saline (50 U heparin included) through vena dorsalis penis. After depilating thoracic and abdominal furs with sodium sulfide (8%) and double sterilization with iodophors, the abdominal wall was then opened through a cross-shape incision, with the transvers incision being at the epigastrium while the longitudinal one in the middle of abdomen. Keeping liver immobile, the inferior vena cava below the liver was liberated till its branch, i.e., left renal vein, before ligating the right renal vein with 10-0 silk thread. After that, the portal vein was separated till its third tributary, with the first tributary untreated but the second ligated. Then a wedge-shape incision was conducted to the common bile duct, through which a stand tube was placed in and ligated with 10-0 silk thread. Starting at the coeliac trunk, the left gastric artery, the splenic artery and the gastroduodenal artery were sequentially liberated, ligated and abscised, leaving the common hepatic artery untreated. The abdominal aorta was then liberated and ligated at the place above the coeliac trunk with No. 1 thread before infusing 4 ml heparin saline (500 U heparin included) into the inferior vena cava at the segment that inferior to the liver. Immediately after that, the lower segment of abdominal aorta was punctured and pumped into 20 ml heparin saline at a rate of 150 ml/h and a temperature of 4°C through a YBWZ-12 micro-infusion pump (Shanghai Three Peak Edward Electric Co., Ltd, Shanghai, China). Taken as outlet of the aforementioned infusion, incisions were made on the inferior vena cava both at its thoracic segment and at the level of left renal vein. With 4°C saline continuously flushing its surface, the liver was then separated and took out quickly after cutting off plexus venosus, ligaments around the liver, the first and second tributaries of portal vein, the portal vein at the place of its third tributary, the vena phrenica as close as possible to the liver, the inferior vena

Improved method for heterotopic auxiliary liver transplantation

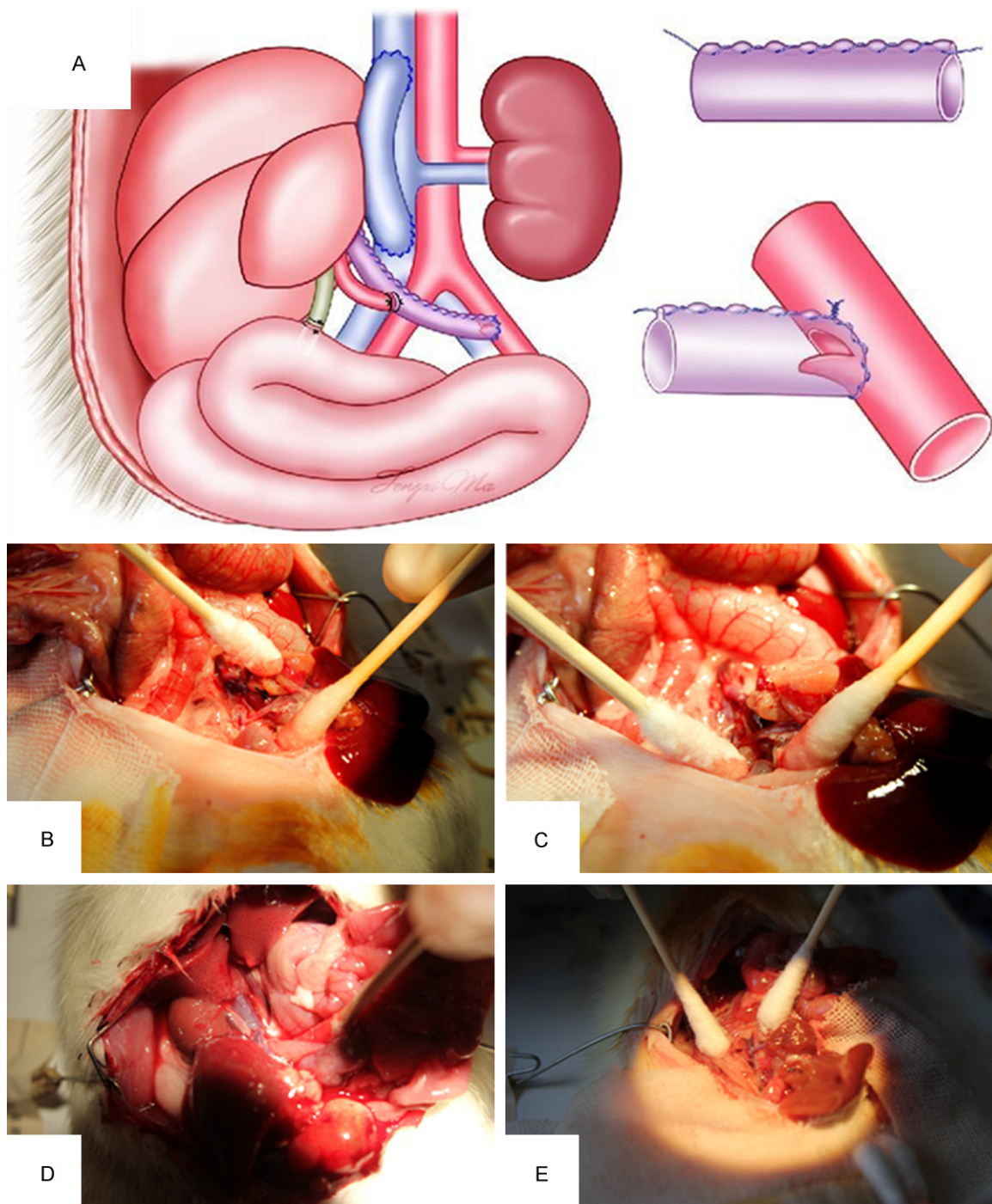


Figure 1. Diagrammatic sketch and intraoperative photos of HALT-PVA. A: Diagrammatic sketch showing the improved heterotopic auxiliary liver transplantation with portal vein arterialization; B: Intraoperative photo showing the end-to-side anastomosis between portal vein of the donor liver and recipient left common iliac artery; C: Intraoperative photo showing a stand-based hepatic artery reconstruction of donor liver; D: Intraoperative photo illustrating end-to-side anastomosis between the inferior vena cava superior to the donor liver and the recipient inferior vena cava; E: Intraoperative photo illustrating end-to-side anastomosis between the inferior vena cava inferior to the donor liver and the recipient inferior vena cava.

cava both at 2-3 cm above the diaphragm and at the level of left renal vein, and the common

hepatic artery in which a stand had been placed and fixed firmly beforehand.

The incised liver was then placed in heparin saline with temperature of 4°C on a flat plate of 10 cm in diameter for liver repairing. For the sake of better vascular anastomosis, the ends of the inferior vena cava both above and below the liver, as well as of the portal vein, were evenly trimmed, and the abundant fatty tissue was removed. Then the stand common hepatic artery was end-to-end ligated to the first tributary of the portal vein with double 10/0 thread, in order to restore blood flow of the common hepatic artery. Opposite to the first tributary of the portal vein, 1/3 of the fringe on the end of the portal vein was stitched up in a spiral, continuous, foldaway and single-line way, with 10-0 prolene thread. The left lateral and middle lobe of the liver (about 70% of the total liver) were resected with the fringe ligated with 9-0 silk thread, leaving around 30% the liver for transplantation, which was stored in 4°C fridge.

For liver transplantation, the anesthesia, depilation and sterilization for recipient rats were same as those for donor rats, which have been described above. All rats were given 0.03 mg atropine intravenously 0.5 h before operation. A longitudinal incision in the middle of abdomen was used to open the abdominal wall. Retractors were used to enlarge the incision appropriately, if necessary, while Bulldog clamps were used to turn the xiphoid process over towards head. During the entire operation, intestine was covered with gauzes which were rinsed with saline beforehand. Before transplantation, 30% of the left lateral lobe of the recipient liver was resected, with 9-0 silk thread ligating the incision margin.

Then, the inferior vena cava of the donor rat from the lower margin of liver to the level of right renal vein, and a segment of 2-3 cm long from the left renal vein downward were liberated. By going downwards alongside the abdominal aorta, the left common iliac artery was found and separated with a distance of 1-2 cm. For sake of temporary blocking the blood flow, a 9-0 silk thread was placed under each segment of these liberated vessels. After being ligated through a slipknot, the left common iliac artery was sheared in a wedge shape to form a valve orifice. The donor liver was then placed into the right paracolic sulci of recipient rat and its portal vein was end-to-side anastomosed to the valve orifice of the left common iliac artery by

using 10-0 prolene thread in a continuous and single-line way of stitch, with the valve embedded anastomotic stoma. The high pressure of blood flow was avoided to infuse too quickly from artery to the portal vein, and thereby decreasing the injury to the donor liver. Subsequently, both ends of inferior vena cava up and below the donor liver were correspondingly end-to-side anastomosed to the segments of recipient inferior vena cava which had separated beforehand as mentioned above.

When all vascular anastomosis were completed, ligations on the recipient inferior vena cava were firstly unleashed in order to open outlets. Then, ligations of the left common iliac artery were slowly loosened from the distal one to the proximal one, by which, acute temporary liver injury caused by hyper-perfusion of hyper-pressure blood from the artery could be avoid. Finally, hepaticocenterostomy were done to all rats. Of note is that, all stitches cannot be too dense and all ligations cannot be too tight, ensuring that the blood flow free at the anastomotic stoma, bringing down the risk of thrombosis. Besides, continuously flushing the anastomotic stoma with heparin saline in order to avoid blood coagulation and poster-operative thrombosis. Pulsations of the portal vein and the common hepatic artery of the donor liver were observed immediately after vascular reconstruction in all rats. Stoma bleeding was not identified in any cases. **Figure 1** provided the diagrammatic sketch and intraoperative photos of IHALT-PVA.

After liver transplantation, warm normal saline with antibiotics (cefuroxime sodium) was given to flush the abdominal cavity, helping rats to regain body temperature and avoid poster-operative infection. After checking that there was no active bleeding, the abdominal wall was closed and stitched up layer by layer. All rats were allowed to drink 0.5 h after operation and to eat 12 h after operation.

PVA model building up for rats from control group

The processes of donor liver excision for rats from the control group were same to those for rats from the experimental group, except that inferior vena cava either superior or inferior to donor liver were stitched up, and that the first tributary of portal vein was ligated. The courses

Table 1. Comparison of serological ALT, AST, Tbil and CHE of rats between the experimental and the control groups at different time points (mean $\pm$ standard deviation)

Items	Group	n	1 d	3 d	5 d	7 d	14 d
ALT	Experimental	3	2160 $\pm$ 22.91	1019 $\pm$ 136.45	62.67 $\pm$ 24.02	37.33 $\pm$ 4.04	39.67 $\pm$ 4.04
	Control	3	2783 $\pm$ 94.63	2165 $\pm$ 183.59	1698 $\pm$ 94.98	874 $\pm$ 110.51	214 $\pm$ 25.61
P-values			0.012	0.013	0.000	0.006	0.005
AST	Experimental	3	5067 $\pm$ 120.07	3293 $\pm$ 151.69	183 $\pm$ 44.79	131 $\pm$ 24.01	162 $\pm$ 12.30
	Control	3	7414 $\pm$ 399.35	5795 $\pm$ 134.92	5302 $\pm$ 157.67	2861 $\pm$ 89.90	364 $\pm$ 21.73
P-values			0.005	0.000	0.000	0.001	0.002
Tbil	Experimental	3	3.83 $\pm$ 0.23	3.60 $\pm$ 0.20	1.67 $\pm$ 0.25	3.57 $\pm$ 0.87	2.73 $\pm$ 0.45
	Control	3	5.23 $\pm$ 0.15	12.17 $\pm$ 1.58	8.70 $\pm$ 1.10	8.53 $\pm$ 0.76	4.80 $\pm$ 0.30
P-values			0.031	0.013	0.012	0.027	0.013
CHE	Experimental	3	63 $\pm$ 1.35	74.67 $\pm$ 4.93	62.73 $\pm$ 3.22	86.13 $\pm$ 9.10	115 $\pm$ 6.66
	Control	3	59.67 $\pm$ 2.52	69.07 $\pm$ 1.05	57.33 $\pm$ 3.51	39.33 $\pm$ 2.51	198 $\pm$ 27.43
P-values			0.116	0.212	0.090	0.020	0.026

ALT, alanine aminotransferase; AST, aspartate aminotransferase; Tbil, total bilirubin; CHE, choline esterase.

of transplanting donor liver to recipient rats for rats from the control group were also mostly same to those for rats from the experimental group, except that the right kidney, adrenal gland and related vascular vessels of the recipient rats were resected and ligated, with the right renal vein being end-to-end anastomosed to the inferior vena cava inferior to the donor liver, and the right renal artery being end-to-end anastomosed to the portal vein of donor liver through a stand.

Biochemical test of liver function

For each transplanted rat, 5 ml blood was collected respectively on 1 d, 3 d, 5 d, 7 d and 14 d after operation through abdominal aorta puncture. Three blood samples of different rats were collected at each time point. Serums were acquired by centrifuging each sampled blood for 10 min at a speed of 3500 rpm, and were sent to biochemical laboratory of the South West Hospital affiliated to the Third Military Medical University for alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (Tbil), and choline esterase (CHE) testing, in order to monitor the change of liver function.

Pathological evaluation

After blood sample collecting, each rat at each aforementioned time point was over anaesthetized to death. All anastomotic stoma were checked for stenosis or thrombosis. After being fixed in formaldehyde (10% in concentration),

dehydrated, embedded in paraffin and sliced, tissues of donor liver were stained with Hematoxylin and Eosin (H&E). The pathological slices were observed on an inverted microscope (BX41, OLYMPUS, Japan) and pictured through the Olympus camera.

Statistical analysis

All statistical analyses were performed using SPSS 19.0 (IBM SPSS Inc., Chicago, IL, USA). Student's *t* tests were performed to identify differences of items of the liver functional tests between the experimental group and the control group. Any difference with $P < 0.05$ was defined as significant. Summary statistics were expressed as means $\pm$ standard deviation (SD).

Results

Status of the operated rats

Two rats from the control group died because of per-operative blood loss, while only one rat from the experimental group died due to anesthetic accident, with the death rate being 13.3% (2/15) and 6.7% (1/15), respectively. These three rats were excluded from final statistic analysis. The rest rats survived and moved freely 1-2 h after operation, and then began to drink water and take food.

Biochemical results of donor livers function

Biochemical data of donor liver functional test were summarized in **Table 1**. The serological

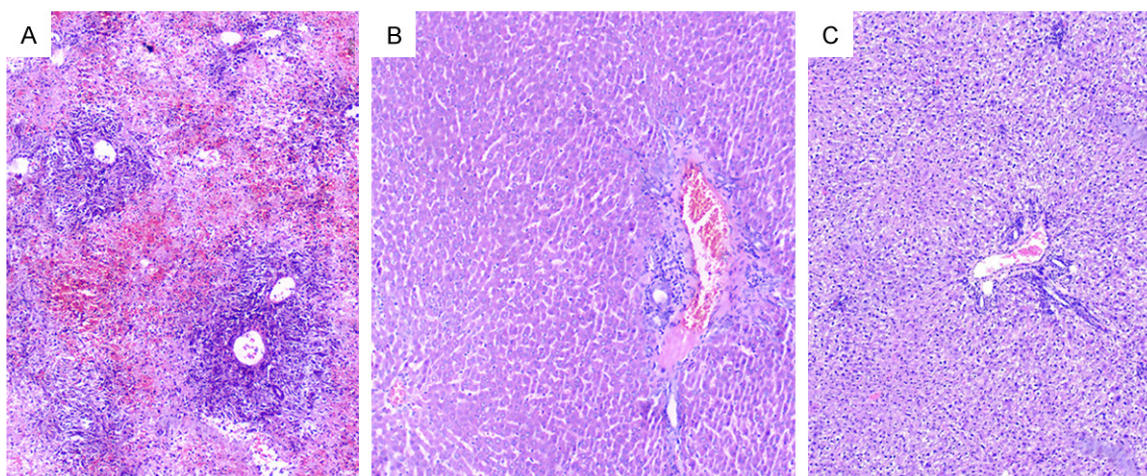


Figure 2. Images of pathological sections (H&E staining) of liver tissues from the control rat (A) and the experimental rat (B) sampled on the 14th day after operation, as well as from the normal rat (C). (A) In liver tissue of rats from the control group, disorders structures of hepatic lobules, central veins and portal areas can be observed with apparent dilation and congestion in the central vein, infiltration of inflammatory cells, as well as obvious vasodilation and hyperemia in the peripheral liver sinusoids. Along these sinusoids, enlarged endothelial cells with huge nuclear and red-stained cytoplasm are shown. Numerous hepatic cells present cell shrinkage, with nuclear decreases, pyknosis and necrosis could be observed. (B) In liver tissue of rats from the experimental group, distinct hepatic lobule, central vein and portal area can be observed, with mild dilation and congestion in the central vein, unapparent infiltration of inflammatory cells, as well as vasodilation and hyperemia in the peripheral liver sinusoids. Along these sinusoids, enlarged endothelial cells with huge nuclear and red-stained cytoplasm can be identified. Some hepatic cells present cell shrinkage, with nuclear decreases, pyknosis and necrosis can be observed. In the portal vein, infiltration of a few inflammatory cells as well as mild dilation and hyperemia in the interlobular vein can be found; while around the portal area, regenerated hepatic cells with red-stained cytoplasm, enlarged cell size and apparent dikaryon are shown. (C) Normal liver tissue of rat shows distinct hepatic lobule, central vein and portal area, with no infiltration of inflammatory cells, normal endothelial and hepatic cells.

ALT, AST and TBil tested 1 d after operation reached peak values, and showed a significant decreasing trend in the following days in rats both from the experimental group and the control group. In rats from the experimental group, these three items decreased more quickly which almost reach to the normal levels, compared to those in the control group, which continuously remained at a high level (P values all less than 0.05). The CHE tested on the 1 d, 3 d, 5 d and 7 d postoperatively were all lower than the normal range, either in the experimental group or in the control group. As CHE is mainly synthesized in liver, its descent in serological concentration commonly indicates hepatocellular injury. As an important item in evaluating hepatocellular injury and an index in assessing severity of hepatic diseases as well as their prognoses, the serological CHE tested on the 14th day postoperatively rose back to the normal range in rats from the experimental group, whereas those in rats from the control group rose even notably to had surpassed the normal range, meaning the injured hepatic cells in rats

from the control group restored more difficult, compared to the experiment group.

Pathological findings

As compared to the control group (**Figure 2A**), the hematoxylin and eosin (H&E) staining of the liver tissue from the experimental group (**Figure 2B**) performed on the 14 d postoperatively showed distinct hepatic lobule, central vein and portal area, with mild dilation and congestion in the central vein, unapparent infiltration of inflammatory cells, as well as vasodilation and hyperemia in the peripheral liver sinusoids. Along these sinusoids, enlarged endothelial cells with huge nuclear and red-stained cytoplasm were observed. Some hepatic cells presented cell shrinkage, with nuclear decreases, pyknosis and necrosis could be observed. In the portal vein, infiltration of a few inflammatory cells as well as mild dilation and hyperemia in the interlobular vein could be identified; while around the portal area, regenerated hepatic cells with red-stained cyto-

plasm, enlarged cell size and apparent dikaryon could be found. However, all these changes were not obvious compared to the pathological presentation of normal hepatic tissue (**Figure 2C**).

Discussion

Heterotopic auxiliary liver transplantation with PVA (IHALT-PVA) has special advantages in clinical application. First of all, the blood supply of the recipient liver is intact, which means the competition of portal vein blood supply between the recipient liver and the donor liver will not exist, while sufficient blood provision to the portal vein can, in turn, overcome weak point of high pressure of blood flow at the outlet [3, 9]. Thus, the donor liver is easy to be transplanted into and excised from the recipient. Furthermore, through IHALT-PVA, portal vein thrombosis of donor liver can be avoid, indicating that IHALT-PVA offers an alternative way of liver transplantation for those whose portal vein reconstruction are unavailable.

In this study, some improvements to IHALT-PVA in position selection of donor liver and in vascular reconstruction were carried out. The method of liver transplantation presented in this study complies well with the normal anatomy, and is more stable and operative. Thus, it may exhibit great values in clinical application, due to several improvements or advantages of the method presented in this study:

Firstly, the position for donor liver is paracolic sulci, which is more spatial than the conventionally used renal region, sub-hepatic area, splenic fossa, and iliac fossa, etc. This leads to a better vascular reconstruction of donor liver and less compression to surrounding organs. Besides, when the rat turn over poster-operatively, the diaphragmatic facies of the donor liver will stick closely to the abdominal wall, avoid twist and thrombosis.

Secondly, to achieve a complete PVA, the vascular reconstruction for donor liver was carried out through end-to-side anastomosis between the valve orifice of left common iliac artery and the lateral wall of the portal vein which had constricted a little bit by a spiral, continuous, fold-away and single-line way stitch. To our experience, constriction simply proximal to the stoma, aiming to avoid arterial blood overflow into the

portal vein, may lead to vortex in the relatively wide trunk of the portal vein and thereby causing thrombosis [16]. As compared to the model advocated by Karina Schleimer [12, 14], of which the right kidney was resected and the corresponding artery was end-to-end anastomosed to the portal vein of donor liver through a stand, the method in current study is harmless, better comply with normal anatomy and the transplanting ethics, and bring up survival rate and living quality, as it do not need to 'sacrifice a kidney'. Above all, without needing any xenobiotic stand for vascular anastomosis, the reconstructed portal vein and inferior vena cava in this study will not cross or compress to each other, which will, to a large extent, reduce thrombosis. Furthermore, by limiting both the outlet of the high-volume and high-pressure artery blood, and its entrance to the donor liver, the liver will be perfused with appropriate blood pressure and appropriate blood volume, which is helpful in avoiding occurrences of complications, such as liver hyper-perfusion, liver rupture hemorrhage, and so on [13]. Perfusing the donor liver with arterial blood that affluent in oxygen can immediately finish anhepatic phase, and makes the metabolism and regeneration of donor liver more efficient [10, 11, 18], benefiting the survival time after hepatectomy. Thus, PVA can be taken as common treatment to patients with poster-hepatectomy liver failure [15].

Thirdly, hepatic arterial reconstruction for donor liver was accomplished through a stand based anastomosis between the hepatic artery and the first tributary of portal vein with an acute angle, by which sufficient arterial blood can infuse into the reconstructed hepatic artery through the arterialized portal vein trunk. This is conducive to maintain the normal hemorheology, bring down the ischemia of bile duct after liver transplantation, protect bile canaliculus, reduce bile duct related complications and hepatic impairment, and even improve long-time survival rate after the operation [2, 4, 6, 17, 20].

Fourthly, reconstruction for the inferior vena cava both superior and inferior to the donor liver was carried out via single-line continuous stitch of end-to-side anastomosis between the two ends of inferior vena cava (donor liver) with the recipient inferior vena cava achieved abso-

lute flow free at the outlet of the donor liver, as compared to the model that stitch up the superior end of donor inferior vena cava while only anastomose the inferior end. The free flow at the outlet of the donor liver may also alleviate portal hypertension and decrease the risk of thrombosis alike complications [19]. Most importantly, it helps to maintain stable hemodynamics for donor liver, which is of great importance to the regeneration of donor liver.

Finally, only 30% of the donor liver was used in the model of this study. The lower requirement in quantity of donor liver makes the current model more practicable, given the status of scarce supply of donor liver.

Conclusion

The improved heterotopic auxiliary liver transplantation with portal vein arterialization accords with the normal anatomy even better, which has potential to improve the survival rate and living quality of recipient, and, therefore, is a good model for related experimental research. Besides, this study also offers powerful technical and theoretical support for its future clinical application. Future studies focus on establishing the blood flow standard for portal vein arterialization, in order to avoid acute blood reperfusion injury and necrosis of liver caused by high-volume, high-rate and high-pressure liver blood supply reconstructed.

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Disclosure of conflict of interest

None.

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