Extended ligation of hepatic vein may obtain similar effect to live venous deprivation in rat model

Xiaoqin He
Renmin Hospital of Wuhan University

Yuefeng Zhang
Renmin Hospital of Wuhan University

Gaoshuo Zhang
Renmin Hospital of Wuhan University

Peng Ma
Renmin Hospital of Wuhan University

Liangkun Xiong
Renmin Hospital of Wuhan University

Wei Wang
Renmin Hospital of Wuhan University

Yangtao Xu
Wuhan University

Yang Shen
Wuhan University

Kaihuan Yu
Renmin Hospital of Wuhan University

Weixing Wang (✉ Wangwx@whu.edu.cn)
Renmin Hospital of Wuhan University

Research Article

Keywords: liver venous deprivation, hepatic vein ligation, hypertrophy, animal model

Posted Date: December 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2363881/v1

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Abstract

**Aims:** To verify the hypothesis that the hepatic vein ligation (HVL) alone may produce similar results to the liver venous deprivation (LVD or HVL/PVL).

**Methods:** Rats were assigned to 5 groups, the control group; R group: the right median hepatic vein (RMHV) was ligated; M group: the middle median hepatic vein (MMHV) was ligated, RM group: both the RMHV and MMHV were ligated; LVD group: both the right median portal vein (RMPV) and the RMHV were ligated. Liver hypertrophy effect and liver enzymes were determined. The methylene blue staining and retrograde pressurized perfusion assay were performed to observe the hemodynamic changes.

**Results:** The RM and LVD groups exhibited similar significant hypertrophy in the future liver remnants when compared to the control group, and almost no additional hypertrophy effect were observed in the R and M group. A remarkable elevation in serum transaminase levels in both those groups. The methylene blue staining indicated that there are pressured-dependent collaterals between the contiguous drainage areas, the R+MMHV procedures block the outflow of RML.

**Conclusion:** The extended ligation of hepatic vein (R+MMHV) obtained the similar hypertrophy effect and hepatic damage to the LVD in rat model, and the intrahepatic venovenous collaterals play key roles.

1. Background

It is well known that sufficient future liver remnant (FLR) is a necessary condition for curative hepatectomy. When faced with the inadequate of FLR, surgeons can choose a variety of methods to gain the FLR. At present, the commonly applied liver preparation procedures include portal vein embolization or ligation (PVE or PVL), associating liver partition and PVL for staged hepatectomy (ALPPS), and liver venous deprivation (LVD) and their variants. However, the advantages and limitations of these methods need to be further studied. Among them, LVD was introduced by Boris Guiu’s team and immediately attracted much attention in recent years. LVD is an effective and safe method that simultaneously combines both portal and hepatic vein embolization (HVE) during the first stage operation. The right portal vein embolization (PVE) using a lipiodol-glue mixture through transhepatic access under ultrasound guidance, and the right hepatic vein embolization (HVE) was achieving by placing an vascular plug at the distal part of the hepatic vein after ultrasound puncture.

In fact, HVE was used to be performed after the PVE to facilitate the proliferation effect of PVE. Many mechanisms have been testified to involve in liver hypertrophy induced by LVD, one widely recognized mechanism is the redistribution of the portal blood flow to the non-injured liver. In a simplified liver model, for the PVE procedure, the interrupted portal blood flow can directly increase the portal vein pressure, consequently, increases the portal blood flow into the contralateral liver lobe. In theory, obstruction of the hepatic outflow can also lead to regurgitation into the portal vein through the
sinusoids, which also can indirectly increase the portal pressure and raise the perfusion of portal blood to the non-embolized lobe. Therefore, we propose a hypothesis that the HVE alone or LVD (HVE+PVE) may produce similar results. After all, the HVE is much simpler than LVD in technique. In this study, the rat model of LVD is developed with the method described in our previous study. Then compare the hepatic hypertrophy effect and liver damage between HVL and LVD (HVL/PVL).

2. Materials And Methods

2.1 Animals

All procedures and housing of the animals were performed in accordance with Chinese animal regulations and guidelines. This study was approved by the Ethics Committee of the Animal Experiment Center of Wuhan University. Male Sprague–Dawley (SD) rats of ~ 350g from Hunan SJA (Changsha, China) were housed under special pathogen-free conditions with controlled temperature (23°C) and a light/dark (12 h:12 h) cycle. Animals were provided with standard rat food and water. These surgery were conducted after a week adjustment period.

2.2 Experimental design

The LML were designed as the FLR in the experiment groups. Specifically, a total of 60 rats were randomly assigned to 5 groups (n = 12, each group, Table 1 & Fig. 1D), the control group; R group: the right middle hepatic vein (RMHV) was ligated; M group: the middle middle hepatic vein (MMHV) was ligated, RM group: both the right and middle middle hepatic vein (R + MMHV) were ligated; LVD group: both the right hepatic vein (RMHV) and the right middle vein (RMPV) were ligated. Due to the portal vein branch supplying the caudate lobe is too small to dissect, caudate lobes of all the rats were removed. In addition, because the left lateral lobe (LLL) and right lateral lobes (RLL) account for a large proportion of the whole liver, the portal vein supplying LLL and RLL was ligated, respectively.
Table 1
Experimental designation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Removal of the caudate lobe</th>
<th>Ligation of the PV supplying the RLL and LLL</th>
<th>Ligation of the MMHV</th>
<th>Ligation of the RMHV</th>
<th>Ligation of the RMPV</th>
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<tbody>
<tr>
<td>Control</td>
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<td>LVD</td>
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</table>

“+” indicates “yes”; “-” indicates “no”. **Abbreviations:** LLL, left lateral lobe; LVD, liver venous deprivation; MMHV, middle median hepatic vein; RLL, right lateral lobe; RMHV, right median hepatic vein; RMPV, right median portal vein.

2.3 Anesthesia and surgical procedures

Animals were anesthetized by inhalation of a mixture of 3% isoflurane (RWD, Shenzhen, China) mixed with pure oxygen at a flow rate of 0.5 L/min. Shaved the hair and disinfected the abdomen skin, then made a 5.0 cm midline incision. Release the falciform ligament is necessary to fully expose the HVs (Fig. 1B). After the operation, each animal was injected with 6 mL of a 5% glucose and sodium chloride solution containing 0.96 mg of gentamicin. Animals were placed on a heating pad to keep warm before recovery.

**PVL**

The RPV was ligated or sutured with 5−0 nylon thread. And avoid damaging the accompanying bile duct and artery during this procedure.

**HVL**

The RMMHV is apparent and easily to be dissected and ligated. While for rats, MMHV confluences into the left median hepatic vein (LMHV), and embed in the liver parenchyma. The dissection of MMHV would injure the vena cava or the MMHV and lead to uncontrollable bleeding, so we choose to suture with 5−0 nylon thread, and choosing the right angle and depth was key to the successful suture (Fig. 1C).

2.4 Sample collecting

At POD 2, and 7, the animal (n = 6 at each time point) were sacrificed and blood samples were collected into serum tubes (BD Biosciences, America), and serum was isolated by centrifugation method and stored at -20 ºC. The livers were explanted with no accessory vessels and ligaments. Each individual liver lobe was weighted and recorded. Due to each rat liver lobe compared to the body weight in normal
healthy SD rats is constant, our previous data showed the LML/body weight is about 0.00376, so the LML growth ratio was calculated using the following formula:

\[
\text{Growth ratio} = \frac{\text{Actual LML weight} - \text{Initial body weight} \times 0.00376}{\text{Initial body weight} \times 0.00376}
\]

### 2.5 Liver injury assessment

The serum values were related to the liver functions, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (T-Bil) and albumin (ALB), were examined using an automatic procedure in a serum multiple biochemical analyzer (Siemens, Germany).

### 2.6 Pathological Examination

The harvested liver lobes (LML and RML) were fixed in 4% buffered formalin for 48 hours. After paraffin embedding, these tissues were cut into slide (4 mm thick). Standard hematoxylin-eosin (H-E) staining was conducted to observe ultrastructural changes. Picric acid-Sirius red staining was performed to display liver mesangial collagen fibers. Additionally, Ki-67 (Proteintech, Wuhan, China) immunostaining was performed to evaluate the hepatocyte regeneration. All sections were digitalized with a Slide Scanner (Hamamatsu Electronic, Japan). The number of Ki-67-positive hepatocytes was determined by counting 1,000 hepatocytes in 5 randomly selected visual fields (20×).

### 2.7 Methylene blue staining assay

The methylene blue staining assay was performed to indicate the collaterals between the conterminous drainage area of hepatic vein. Specifically, the different model were completed as described before, 100ul solution of methylene blue were injected into the liver, and pressed needle eye with cotton or closed the needle eye with surgical cautery pencil. The puncture site should be chose at the middle of the drainage basin of hepatic vein. After 30s, dyeing range were observed and recorded.

### 2.8 Retrograde pressurized perfusion assay

Posterior vena cava puncture and hepatic vein catheterization was conducted firstly (Figure S1), and the conduit placed in the RMHV, then RMHV and MMHV were ligated. The methylene blue solution was injected into the RMHV, and dyeing range were observed with the increase of injection pressure.

### 2.9 Statistical analysis

All the quantitative data are presented as the mean values plus standard deviations (mean ± SD). GraphPad Prism 6.0 software (San Diego, CA, USA) was used for graphs and statistical analyses. Multiple t test or Student’s t test was applied to compared the differences between multiple groups or two groups, respectively. The level of statistical significance was set at \( P < 0.05 \).

### 3. Results
3.1 The experiment designation

The RML, LML and their vascular system in rat structurally mimic the human liver, so they were selected as the observed objects, the LML were designed as the FLR in the experiment groups (Fig. 1A & D). As described in Fig. 1A, the RMHV, MMHV and LMHV have their independent and clear drainage area (DA), which were termed as DAR, DAM and DAL, respectively.

3.2 Macroscopic findings

As shown in Fig. 2A, immediately after ligation of RMHV or MMHV in R, M and RM group, an apparent demarcation line shown between the obstruction zone and innocent liver, the area of dark red discoloration indicated the liver territory of HV. While for the LVD group, all the RML firstly turned from fresh red to dull-red after ligation of RMPV, then the DAM turned to dark red after the ligation of RMHV.

At POD 2, the color of congestive area was diminished, the borderlines between the normal and obstructive area were distinguishable and stay at the original positions in all the experiment groups (Fig. 2A). At POD 7, the obstructive zones in R and M groups almost completely recovered to normal, the dividing or marked lines disappeared. The edge of all the liver lobes were blunt, which indicated the regeneration of liver. In contrast, DARs in the RM and LVD groups were shrink, hard, pale and yellow, indicating complete necrosis. The DAMs were atrophied at some extent. Notably, the FLRs (LML) in the RM and LVD groups were hypertrophied (Fig. 2A).

All the rats survived from those surgical procedure, they suffered from fast body loss at the first 2 days after surgery, then gradually recovered (Fig. 2B), which led the liver/body weight ratios reached the summit at POD 2 and then fall (Fig. 2C). The proliferation extent of FLRs (LML) were quantitatively analysis by the growth ratio. As seen in Fig. 2D &E, the LMLs were hypertrophy in the all the groups (LML growth ratio > 1), but the LML/RML ratio in the control, R and M group remain stable or have small change, which implied that the LML and RML generate the same degree of grow, so the regeneration of LML might be spurred by the remove of caudate lobe and PVL of liver right lobes. In contrast, the LML in the RM and LVD group were significantly proliferated when compared to the control group (2.54 ± 0.74, 1.85 ± 0.43 vs. 1.38 ± 0.23, P < 0.05). Moreover, the LML/RML ratio were significantly rise to 2.57 ± 0.53, 2.73 ± 0.49, respectively (P < 0.001). It was suggested the proliferation of LML in the RM and LVD group were provoked by the procedures other than remove of caudate lobe and PVL of liver right lobes. There was no significant difference between the RM and LVD groups at different time-points.

3.3 Microscopic findings

Both the LML and RML lobes in each group were subjected to tissue microarray analyses (Fig. 3). As shown in Fig. 3A, enlarged and crowded hepatocytes were observed in the both these groups, except the NC group. The liver regenerative responses were measured with the staining of Ki-67, a classical marker
for cell proliferation. Apparently, the expression of Ki-67 in the RM, LVD group were significantly higher than that in control, R, M groups POD 2 (73.57 ± 8.88, 74.23 ± 7.67 vs. 15.40 ± 2.83, 37.05 ± 11.16, 42.54 ± 8.52, P<0.001), and there no was different between the RM and LVD group (P>0.05).

Regarding the pathological changes in the LML in experiment groups, large areas of confluent pericentral necrosis and scattered viable portal hepatocytes-islands were observed in the R, M, RM and LVD groups on POD2 (Fig. 3B). Surprisingly, the initial obstructive area was almost recovered, only little scar tissue and fibrosis were found in the R and M groups on POD7, as same as the DRM in the RM group. In contrast, DRM was replaced with fibrosis tissue in the LVD group (Fig. 3B). These results suggested the congestive lobes in the LVD group suffered from more complete and severe necrosis as well as scar repairing.

3.4 Liver functions

The serum ALT, AST, T-Bil and ALB levels were examined to reflect the impairment of liver function. Both ALT and AST levels were elevated in control, R, M, RM and LVD on POD2 (162.50 ± 107.99 vs.149.33 ± 93.19, 236.50 ± 67.44, 254.50 ± 223.76, 217.00 ± 74.33 U/L, P>0.05; 567.00 ± 198.81 vs. 395.17 ± 120.13, 709.33 ± 415.28, 614.33 ± 475.52, 650.83 ± 250.07 U/L, P>0.05), and they returned within normal values on day 7 (Fig. 4A&B). T-Bil levels moderately increased after surgery (2.03 ± 1.26 vs. 3.98 ± 5.51, 3.32 ± 2.75, 3.63 ± 3.03, 3.55 ± 1.86 umol/L, P>0.05) and declined on POD7 (Fig. 4C). Finally, ALB levels exhibited the opposite trend and declined to 29.67 ± 1.59g/L and 27.50 ± 3.09 g/L in the RM (31.26 ± 0.95g/L, P>0.05) and LVD (31.26 ± 0.95g/L, P<0.05) groups, respectively. The ALB levels in RM and LVD group did not return to normal even on POD 7. No significant difference was observed between the RM and LVD groups at different time-points.

3.5 Methylene blue staining

The dyeing range of methylene blue could mark the flow direction of blood outflow (Fig. 5A). In the control group, the blue dye was injected into the DAR and DAM, respectively. It was observed that the blue dye immediately flow to the direction of HV, and hardly gone across the corresponding drainage area. After the ligation of RMHV or MMHV, the methylene blue flow from the obstructive areas to the contiguous normal area. This phenomenon suggested that there exist bi-directional opening bilateral between the two adjacent drainage areas. Interestingly, after the ligation of both RMHV and MMHV, the injected blue dye could flow from DAM to the DAL, but hardly flow from DAR to the DAM.

The opening of bilateral might be pressure controlled, so the retrograde pressurized perfusion assay was performed (Fig. 5B). With the increase of injection pressure, the DAR was first dyed with methylene blue, then hepatic-portal venous reflux was observed, then the undisturbed liver lobes, including LLL, LML and RL, turned blue, while the DAM kept the dull red appearance. In order to further increase the pressure of
RMHV, the hepatic pedicle was occluded with vascular forceps. Continue to push the methylene blue, the total RML were apparently swelling and enlarged, and part of DAM were dyed with methylene blue.

4. Discussion

LVD is a novel technique to increase FLR, and many clinical studies have proved that it is more effective than PVE\textsuperscript{10-15}. However, the mechanism of liver hyperplasia caused by LVD has not been clearly elucidated\textsuperscript{4,5}. At present, the popular view on LVD was that PVE can occlude the inflow of portal vein, besides, HVE restricts the inflow of hepatic artery by blocking the blood outflow and mitigate the arterial buffer effect led by PVE, as a result, more blood of the portal vein and hepatic artery was redistributed to contralateral liver\textsuperscript{2-5}. In fact, the pressure of portal vein is significantly lower than that of hepatic artery (6 ± 2 vs. 110 ± 20 mmHg in human, 7.8 ± 1.1 vs. 124 ± 18 mmHg in rat\textsuperscript{16,17}), according to above view, HVE could also strongly limit the inflow of portal blood if HVE could restricts the inflow of hepatic artery. As analogies go, could HVE alone achieve the same effect as LVD?

In fact, the sometimes the sacrifice of major hepatic vein was inevitable in hepatic segmentectomy, hepatic trauma or adult-to-adult living donor liver transplantation, though the liver function per unit volume in the hepatic veno-occlusive area was approximately 40% of that in the unaffected areas at early stage, serious atrophy or necrosis do not frequently occur in the preserved segment\textsuperscript{18-21}. Furthermore, both the our and previous rat experiments have also found that the RML could almost completely restore to normal state after ligation of RMHV alone on POD 7, and there was no obvious hyperplasia in other liver lobes\textsuperscript{22}. In a word, liver showed good tolerance for the regional obstruction of hepatic vein. While the proliferation of FLR was always accompanied with the lesion of other lobes, obviously, the occlusion of only one HV alone cannot induce the hypertrophy of contralateral liver.

However, whether the ligation of two HV branches at the same time could result in damage of the congested area and stimulate the proliferation of no-congested area? As shown in Fig. 1&2, it was observed that occlusion of RMHV or MMHV alone did not cause atrophy of occlusive area, which was consistent with the previous experimental results. In contrast, the extend HVL resulted in the necrosis of part of RML and contributed to the proliferation of FLR, which can achieve the same hypertrophy effect of LVD. In addition, extended HVL and LVD exert a more serious damage on liver function than other procedures (Fig. 3&4). However, there was no significant difference between the RM and LVD groups (Fig. 3&4).

Next, the methylene blue staining experiment was carried out to investigate the mechanism behind the above phenomenon. As shown in Fig. 5A, the blue dye could flow from congestive areas to un-congestive areas when only one HV was ligated, which indicated that there exist intrahepatic venovenous shunts in the two adjacent drainage areas. The intrahepatic venovenous bilateral have been found in human or animal for a long history\textsuperscript{23}. This assay indicated the two characteristics of these collateral pathways: 1. Two-way opening, blood always flow from the congestion area to the normal area without special
direction; 2. Real-time opening (Fig. 6). The opening of the collateral can be detected within a few seconds after ligation of HV.

However, it was also noticed that methylene blue could not flow between two adjacent congestive areas when both the RMHV and MMHV were ligated, which implied that the intrahepatic venovenous pathway between DAM and DAR were closed. Apparently, the occlusion of intrahepatic venovenous pathway aggravated the blood stasis, eventually led to the necrosis of DAR in RM group. The reason for the closure of venovenous pathway needed to be further investigated. A hypothesis was put forward: the opening of venovenous collateral is pressure dependent. When the pressure difference between the congestive and non-congestion area exceeds the threshold, so as to make the traffic branches open. While the pressure gradient between two congestion areas was too low to open the bilateral. Then the compression experiment was conducted to prove the above hypothesis. As expected, with the pressure of RMHV ascend, methylene blue in the drainage area of RMHV begins to flow to the drainage area of MMHV (Fig. 5B).

This experiment preliminarily indicated that the extended-HV occlusion may obtain the similar effect to LVD in rat model and have great significance in developing new technique to increase FLR. According to previous reports, LVD was performed by ultrasound-guided liver puncture to complete PVE and HVE. In fact, this operation is technically difficult and needs experienced doctors to complete; Moreover, there is a great risk of bleeding, because after LVD, part of the liver is congested and swollen. Bleeding at the liver puncture point is almost inevitable if the puncture point is in the congested area. This was fully observed when injected methylene blue into the congestion area. However, the implementation of HV occlusion can be completed by radiation intervention, intubation through internal jugular vein or femoral vein, and then placing the vascular plug at the HV. Extended HVE is simpler then LVD in technique, which may be easy to popularize, and avoids the risk of liver bleeding.

In this experiment, the intrahepatic venovenous shunt was observed in almost all the rats. While there was only 36.3% of patients(94/259)with varying degrees of intrahepatic venovenous shunt when examined the wedged hepatic venous pressure patients with cirrhotic portal hypertension. In fact, other research have found that intrahepatic venovenous shunt occurred less frequently in cirrhotic patients, which may explain the difference between cirrhosis patients and healthy rats. However, it was also implied that the cirrhosis and fibrosis in liver may destruct the normal tissue structure as well as the venovenous collaterals.

There exist some limitations to present study. Firstly, the structure of animals may be different from that of human, and the conclusions of this study may not be fully applicable to human body. Secondly, the opening of venovenous collateral in the early stage was pressure excited, while the stable intrahepatic venovenous shunt was confirmed in LVD pig model, so the development of intrahepatic venovenous shunt in long termed should be further studied. Last but not least, the intrahepatic venovenous shunt is an important outflow channel for the non-isolated liver lobe, and it is conditionally open. Different treatments targeting the liver inflow / outflow pathway can have different effects on the liver, as shown in
Table 2. so what the effects of those different procedures on the pressure of HV and the formation of intrahepatic venovenous shunt? The role of intrahepatic venovenous shunt play in should be further investigated.

Table 2

Different treatments targeting the liver inflow / outflow pathway can have different effects on the liver.

<table>
<thead>
<tr>
<th>Procedures</th>
<th>Microscopic changes of congestive liver lobes</th>
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<td>in short term</td>
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<td></td>
<td>in a long term</td>
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<tr>
<td>HVL (RMPV)</td>
<td>Necrosis and viable portal islands</td>
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<tr>
<td>Extend HVL (R + MMPV)</td>
<td>Necrosis and viable portal islands</td>
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<tr>
<td>HVL + PVL (RMHV + RMPV, LVD)</td>
<td>Necrosis and viable portal islands</td>
</tr>
<tr>
<td>HVL + HAL (RMHV + RA)</td>
<td>Necrosis and no viable portal islands</td>
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</table>

Abbreviations: DAM, drainage area of middle median hepatic vein; DAR, drainage area of right median hepatic vein; HVL, hepatic vein ligation; HAL, hepatic artery ligation; LLL, left lateral lobe; LVD, liver venous deprivation; RMHV, right median hepatic vein; RMPV, right median portal vein; PVL, portal vein ligation;

5. Conclusions

In conclusion, the rat experiment proved that the extended HVL can achieve the same results as LVD (HVL + PVL). In addition, expanded HVE procedure eliminated the pressure difference two adjacent hepatic vein drainage areas, prevent the formation of intrahepatic venovenous shunt, which more completely block the blood outflow of congest area, resulting in the necrosis of the liver lobe and the proliferation of the contralateral liver. There are three characteristics of general branch: 1 Two way opening; 2. Timely opening; 3. Pressure-dependent. Finally, the operation of expanded HVE is simpler than LVD, with less risk of bleeding and greater clinical significance, but more clinical trials are needed.

In conclusion, the rat experiment proved that the extended HVL can achieve the same results as LVD (HVL + PVL). In addition, expanded HVE procedure eliminated the pressure difference two adjacent hepatic vein drainage areas, prevent the formation of intrahepatic venovenous shunt, which more completely block the blood outflow of congest area, resulting in the necrosis of the liver lobe and the proliferation of the contralateral liver. There are three characteristics of general branch: 1 Two way opening; 2. Timely opening; 3. Pressure-dependent. Finally, the operation of expanded HVE is simpler than LVD, with less risk of bleeding and greater clinical significance, but more clinical trials are needed.
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**Abbreviations**

ALB, albumin; ALT, alanine aminotransferase; ALPPS, associating liver partition and portal vein ligation for staged hepatectomy; AST, aspartate aminotransferase; CL, caudate lobe; DA, drainage area; FLE, future liver excised; FLR, future liver remnant; GS, glucose and sodium chloride; H-E, hematoxylin-eosin; HV, hepatic vein; HVE, hepatic vein embolization; HVL, hepatic vein ligation; LLL, left lateral lobe; LMHV, left median hepatic vein; LML, left median lobe; LMPV, right median portal vein; LVD, liver venous deprivation; MMHV, middle median hepatic vein; POD, postoperative day; PV, portal vein; PVE, portal vein embolization; PVL, portal vein ligation; RIL, right inferior lobe; RMHV, right median hepatic vein; RML, right medial lobe; RMPV, right median portal vein; RSL, right superior lobe; T-Bil, total bilirubin;

**Declarations**

**Conflict of interest**

The authors have no conflicts of interest related to this article to declare.

**Data sharing statement**

Data and materials are available upon reasonable request to the corresponding author.

**Author contributions**

Study concept and design (XQH, YFZ, GSZ), acquisition of data (PM, LKX, WW), analysis and interpretation of data (YTX, GSZ), drafting of the manuscript (YS), critical revisions of the manuscript for important intellectual content (QXH, KHY), and administrative, technical, or material support, study supervision (KHY, WXW).

**Funding**

This project was financially supported by the Fundamental Research Funds for the Central Universities (NO. 2042020kF0124), the National Natural Science Foundation of China (NO. 82001940) and Hubei province Key Laboratory Opening Project (NO.

**References**


Figures

Figure 1

Schematic of the different rat models.

(A) Portal (left) and hepatic (right) vein anatomy of the RML and LML are displayed.

(B) The second hepatic hilum of rat liver. The posterior vena cava, RMHV and common trunk were presented.

(C) The MMHV were obstructed with suturing ligation, the needle entry and exit points were shown.

(D, E, F, G) Schematics of the R, M, RM and LVD groups are described, respectively.
Figure 2

Liver remodeling after the different surgeries.

(A) Changes in the appearance of the live ML after surgery on postoperative days (PODs) 0, 2 and 7.

(B) Changes in the body weight at each time point.

(C) Weight of the remnant liver/body weight evolution.

(D) The LML growth ratio of different groups were shown.
The LML/RML shows the morphological changes in the ML after surgery.

(*, RM vs. Control; #, LVD vs. Control. * or #, $P < 0.05$; ** or # #, $P < 0.01$; *** or ###, $P < 0.001$).

**Figure 3**

Changes in liver serum enzymes after surgery.

The liver function and injury were evaluated by the serum levels of AST (A), ALT (B), T-Bil (C) and ALB (D).

(*, RM vs. Control; #, LVD vs. Control. * or #, $P < 0.05$; ** or # #, $P < 0.01$; *** or ###, $P < 0.001$).
Figure 4

Histologic and immunohistochemical assessment.

(A) The LMLs were subjected to a tissue chip analysis. Slides were stained with H-E (upper) and Ki-67 (lower). Quantification of the number of Ki-67-positive cells in the LML after surgery in all of the different groups. (*, RM vs. Control; #, LVD vs. Control. * or #, P < 0.05; ** or # #, P < 0.01; ### or ###, P < 0.001).
(B) The RMLs (congestive liver tissues) of the different groups exhibited different changes; thus, the representative images of H&E-staining were presented, respectively. Collagen deposition was detected by Sirius red staining. In the RM and LVD group at POD7, the DAR were necrotic, and DAM were subjected to the histology analysis. The white, red and black arrows indicate the viable hepatocyte islands, necrotic area and fibrous tissues, respectively.

Figure 5

(A)

Control

Ligation of RMHV

Ligation of MMHV

Ligation of both the RMHV and MMHV

(B)

Before occlusion of hepatic pedicle

After occlusion of hepatic pedicle
Methylene blue staining and retrograde pressurized perfusion assay

(A) The RMHV MMHV or both the RMHV and MMHV were obstructed, then injected the methylene blue solution into the center of congested area. The dyeing area indicated the direction of blood flow.

(B) After the occlusion of both the RMHV and MMHV, the methylene blue was retrogradely perfused into the RMHV. These photos presented the liver appearance before (left) and after (right) occlusion of hepatic pedicle, respectively. The hepatic-portal venous reflux was observed and the LLL turned blue (white arrow). After the hepatic pedicle was occluded with vascular forceps and continue to push the methylene blue, part of DAM were dyed with methylene blue (green arrow).

Figure 6

Schematic illustration of intrahepatic venovenous collaterals. There exist several intrahepatic venovenous collaterals between the adjacent hepatic venous drainage areas. This assay indicated the two characteristics of these collateral pathways: 1 Two way opening; 2. Timely opening; 3. Pressure-dependent.

Supplementary Files

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