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Rabbit model provides new insights in liver regeneration after transection with portal vein ligation

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ABSTRACT

Background: The rabbit model of associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) has not been reported before.

Materials and methods: New Zealand white rabbits were allocated to two protocols. Protocol 1 involved either liver parenchymal transection (LPT, $n = 5$) or portal vein ligation (PVL, $n = 5$). Protocol 2 involved the ligation of different portal vein branches combined with liver partition, including the LPT + 20% PVL group ($n = 5$; the caudate portal vein was ligated), the LPT + 50% PVL group ($n = 5$; the left portal vein was ligated), and the LPT + 70% PVL group ($n = 10$; both veins were ligated). Computed tomography liver volumetry was performed immediately after operation. Blood samples were harvested before surgery and at days 1, 3, 7, or 14 after surgery for liver function evaluation. Most rabbits were humanely euthanized on day 7. The livers were harvested, divided into lobes, and weighed; biopsies of each lobe and immunohistochemical staining were performed.

Results: In this article, we present a new rabbit model to simulate ALPPS procedure, with a description of the regional anatomical features, surgical routes, and key techniques. The growth rate of remnant right lobe volume increased with proportionally PVL combined with LPT. Specifically, right lobe volume growth rate of the LPT + 50% PVL group overwhelmed 70% PVL alone.

Conclusions: There were putative underlying mechanisms other than portal inflow redistribution in triggering residual liver regeneration after ALPPS procedure. This rabbit model is feasible for further mechanism research of this special clinical phenomenon.

Introduction

As liver transplantation is limited by organ shortage, surgical resection is the most applicable curative treatment for patients with primary or metastatic tumors of the liver. Accordingly, only ~30%-40% patients have the opportunity of surgical resection, and others are unable to receive radical treatments because of advanced tumor progression or worsened liver function. Thus, in the past decades, the rate of resectability has increased gradually, at pace with surgical

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improvements.[1,2] One of the primary concerns in resecting advanced liver tumors is the risk of liver failure after extensive hepatectomy. Makuuchi *et al.*[3] initially introduced the clinical application of the unilateral portal vein embolization (PVE) technique to enhance liver growth of the opposite lobe, with a waiting time of 4-8 wk to several months.[4-6] Although enlargement of the future liver remnant (FLR) offers critical volume for the patient's postoperative safety, unpredictable risks, such as early tumor progression or inadequate hypertrophy, remain critical concerns for patients' survival. Recently, a special surgical procedure of liver parenchyma transection (LPT) combined with portal vein ligation (PVL), also known as ALPPS (associating liver partition and portal vein ligation for staged hepatectomy), has been introduced as a more efficient strategy to induce rapid liver regeneration.[7]

Clinical interventions revealed that ALPPS has a profound and special role in triggering rapid growth of the FLR, whereas the laboratory researches in underlying mechanisms were just initiated with the modeling procedures. Schlegel *et al.*[8] deftly maneuvered *in situ* splitting with PVL in tiny mouse livers. Impressively, the micro portal branches of the mouse livers were ligated with 9-0 silk under a microscope, which is quite challenging even for an experienced nano-surgeon. The rat liver is a little larger and more feasible for complicated surgeries. After the success of Schlegel *et al.*, several rat models have been reported, with similar surgical procedures but differed in transection portions.[9-12] However, the rat liver was also thoroughly lobulated, only the small median lobe could be split for simulating ALPPS, the anatomic features as well as the small size remains a substantial limitation in the reliability of rat models. In larger animals, Croome *et al.*[13] have previously attempted the complete ALPPS procedure on a porcine model, the velocity of liver growth, and two-stage partial hepatectomy were nearly perfect simulations of the clinical situation. Therefore, the porcine model is more likely to be helpful in surgical technique training than laboratory research of the ALPPS mechanism. Rabbits have been used in previous experiments of liver surgeries, such as portal vein embolism or trans percutaneous arterial chemo embolism therapy. Although rabbits are common laboratory animals, the rabbit model of ALPPS has not been reported.

This study aimed to determine the best protocol using a rabbit model to simulate ALPPS and provide a description of the regional anatomical features, surgical routes, and key techniques.

Methods

Animals

Animal experimentation was approved by the Institutional Ethics Committee of the West China Hospital at Sichuan University, Chengdu, China. Adult male New Zealand white rabbits with a mean weight of 2.56 ± 0.38 kg were fed a standard laboratory diet with water and food *ad libitum* and were kept under constant environmental conditions on a 12-h light–dark cycle. Owing to the aggressive nature of the surgery and the need for consistency of surgical protocols, only male rabbits were adopted. Experiments were carried out in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Experimental design

A total of 30 rabbits were randomly allocated between the two protocols. Protocol 1 involved either LPT ($n = 5$; the liver was split between the left medial lobe [LML] and right lobes [RLs]) or PVL ($n = 5$). Protocol 2 involved three groups of LPT combined with PVL. In the LPT + 20% PVL group ($n = 5$), only the caudate portal vein was ligated; in the LPT + 50% PVL group ($n = 5$), the left portal vein was ligated; in the LPT + 70% PVL group ($n = 10$), both the caudate and left portal veins were ligated. Computed tomography (CT) volumetry was performed immediately after LPT or PVL. Blood samples were drawn before surgery on days 1 and 3 postsurgery, and at euthanasia. Most animals were humanely euthanized on day 7, except for 5 in the LPT + 70% PVL group, which were euthanized on day 14 to evaluate complications, such as bile leakage and liver abscess and long-term liver regeneration. The liver weight was measured, and histologic specimens were collected.

Animals were anesthetized with 30 mg/kg ketamine (Ketalar; Par Pharmaceutical Co Inc, Spring Valley, NY) injected intramuscularly and 2 mg/kg midazolam (Dexdomitor; Orion Corp, Espoo, Finland) intramuscularly together in the same syringe and maintained with a mixture of 1%-3% sevoflurane (Baxter Healthcare of Puerto Rico, San Juan, Puerto Rico) and 50% oxygen and air (0.8-1.5 L/min). Liver surgery was performed by a liver surgeon with more than 5 years of experience. Animals were placed flat on their back in the Trendelenburg position, and after midline laparotomy, the left branch (Fig. 1A and B) and caudate branch (Fig. 1C and D) of the portal vein were precisely dissected and labeled. According to Heath and House,[13] the left portal vein supplies the LML and left lateral lobes, corresponding to 50% of the rabbit liver mass; the caudate lobe accounts for another 20% of the liver volume. Thus, in the PVL procedure, the left portal vein (LPT + 50% PVL group), the caudate portal vein (LPT + 20% group), or both veins (PVL group and LPT + 70% PVL group) were ligated with 5-0 silk (Ethicon, Somerville, NJ). The portal veins of the control group (LPT group) were dissected, but not ligated.

After left portal branch ligation, or in the case of the LPT + 20% PVL and LPT groups, clamping of the left portal branch, the ischemic line between the LML and the RL became prominent (Fig. 1E and F). For groups requiring LPT, the liver parenchyma was then split from the free edge to the anterior wall of the inferior vena cava following the ischemic line (Fig. 1G). Hepatic transection was performed using a 15-mm Harmonic scalpel (Ethicon Endo-Surgery, Cincinnati, OH) connected to a Harmonic Generator 300 (Ethicon Endo-Surgery, Inc) and a bipolar electrode connected to a closed system (Valleylab, Boulder, CO). The devices were assembled and set according to the manufactures' instructions. The Harmonic scalpel was set to a minimum power of 2 W and maximum of 4 W, and the bipolar electrode was set to standard mode with output power of 60 W. Because of concerns over thermal injuries, we used the clamping technique in dissecting liver parenchyma and coagulated the vessels with a small distance from liver tissue.