A preliminary study of associating liver partition and portal vein ligation for staged hepatectomy in a rat model of liver cirrhosis

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Abstract. Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) in a rat model of liver cirrhosis has not, to the best of our knowledge, been previously investigated. The present study therefore aimed to establish a model of ALPPS in cirrhotic rats and to assess liver regeneration. Rats were randomly divided into an ALPPS group with carbon tetrachloride-induced cirrhosis (group A) and a normal liver (group B). Rat weight, cytokine levels, biochemical parameters and histopathology were assessed 1, 2, 3, 7 and 14 days after ALPPS. Higher aspartate aminotransferase and alanine aminotransferase levels were detected in group A on the first postoperative day. On the first, second and third days, hepatocyte proliferation rate was higher in group B than in group A. After 3 days, hepatocyte proliferation rate in group B began to decrease, but the rate in group A continued to increase until the 14th day. Higher levels of hepatocyte growth factor, interleukin-6 and tumor necrosis factor-α were detected in group A compared with group B, but the differences were not significant. The present study demonstrated that ALPPS promoted liver regeneration in a rat model of cirrhosis, but significantly impaired liver function. Compared with the ALPPS model, group B exhibited a delayed peak of proliferation. The mechanism of liver regeneration induced by ALPPS in cirrhotic rats may be associated with increased cytokine levels.

Introduction

The resection of large liver tumors is a frequent intractable problem for liver surgeons (1). A larger hepatectomy can lead to serious complications, including post-hepatectomy liver failure and small-for-size syndrome (2,3). Portal vein ligation (PVL) (4) or portal vein embolization (PVE) (5) have been widely used to increase the future liver volume (FLV) and reduce complication risk of patients with marginal FLVs. Over the 4-8 week waiting period for an adequate FLV, the tumor may continue to progress and as such is a shortcoming of this procedure (5). Associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) has been used for the hepatectomy of large liver tumors since 2007, when Schnitzbauer *et al* (6) described the technique. ALPPS increases FLV in a much shorter time than PVL or PVE (5,7,8). However, according to preliminary reports, this improvement comes at the cost of increased postoperative morbidity and mortality, which justifies further investigation into technique modification (9), particularly in patients with end-stage liver tumors and/or cirrhosis (10,11).

Currently, ~50% of new global liver cancer cases occur in China (12), where hepatocellular carcinoma (HCC) is the third leading cause of cancer-associated death. In China, the prognosis of patients with HCC complicated by liver cirrhosis is poor (13). D'Haese *et al* (10) reported that with strict indications for surgery, patients with liver cancer complicated with hepatic fibrosis may undergo ALPPS surgery. It was also demonstrated that the degree of liver fibrosis and FLV growth rate were negatively correlated (8). However, the precise mechanism and indication of ALPPS in patients with cirrhosis are unclear (10). Research using animal models is therefore required. Current ALPPS animal models in the reported literature are based on normal livers and do not appropriately simulate conditions of liver cirrhosis (14-18). Therefore, the data regarding the feasibility and safety of ALPPS in livers with fibrosis or cirrhosis remains poor. In the present study, an ALPPS model was developed in a highly reproducible animal model of cirrhosis to assess the mechanism of ALPPS, refine the procedure and identify ways to further improve ALPPS outcomes.

Materials and methods

Animal models. A total of 76 male Sprague-Dawley (SD) rats (age, 6-8 weeks; weight, 220-250 g) were obtained from Dashuo Laboratory Animal Co., Ltd. Rats were housed in cages at a temperature of 21-25°C and a humidity of 45-55%. Animals were also exposed to an artificial 12 h light/dark

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cycle with *ad libitum* access to food and water. All procedures were performed according to the guidelines and with the approval of the Animal Care and Ethics committee of the West China Hospital of Sichuan University (Sichuan, China; approval no. 2017001A).

Experimental design. One group of 10 rats (training group) was used to determine basic data which were used to determine normal liver weights and normal liver enzyme range. Rats in the training group received open surgery without liver surgery or model drug injection. After obtaining normal liver tissue samples and serum samples, animals were sacrificed. In the experimental groups, rats were randomly divided into a liver cirrhosis group (group A) and a normal control group (group B). Animals were sacrificed at different time points (1, 2, 3, 7 and 14 days; n=6 animals per group per time point).

Anatomical exploration. The SD rat liver is divided into five sections, which include: The right lobe (RL), the right median lobe (RML), the left median lobe (LML), the left lateral lobe (LLL) and the caudal lobe (CL; Fig. 1A). According to previous experimental studies (17,19,20), liver sections account for the following total liver volumes: LML, 10%; LLL, 30%; RML, 30%; RL, 22%; and CL, 8%. The median lobe is supplied by two portal branches: The right branch and the left branch. This experimental model of ALPPS was developed to maintain the LML, CL and LLL as the FLV (~50%; Fig. 1B-D). Microcomputed tomography and 3-dimensional reconstructions were used to observe individual lobes and hepatic veins (Fig. 1E). Arterial circulation and biliary duct branches were maintained in all rats.

Induction of liver cirrhosis. In group A, liver cirrhosis was induced via a subcutaneous injection of 50% (v/v) carbon tetrachloride (CCl_4 dissolved in olive oil; Chengdu Kelong Chemical Reagent Factory) administered at 1.0 ml/100 g of body weight 2 times a week for a total of 12 weeks as previously described (21). In group B, the same volume of 0.9% sodium chloride solution was injected twice a week for a total of 12 weeks based on rat weight (1.0 ml/100 g). After assessing rat weight, the volume of model drugs was calculated and subcutaneous injections were performed at different sites in the abdomen. On the 1st day of week 13, surgical procedure was performed. Rats were subsequently humanely sacrificed via exsanguination under anesthesia on postoperative days 1, 2, 3, 7 or 14.

Surgical procedure. All surgical procedures were performed under anesthesia with 2-4% isoflurane mixed with pure oxygen at a flow rate of 0.5 l/min (Shenzhen Ruiwode Life Technology Co., Ltd.). Rats underwent a laparotomy via a transverse upper-abdominal incision. The mobilization and dissection of portal veins was performed under an operating microscope (Zeiss GmBH; magnification, x10-25). The branch of the portal vein and branches of the lobes were exposed and prepared for ligation to remove the peripheral ligaments of the liver. A bulldog clamp was briefly applied near the hepatic pedicle (pringle maneuver) (20) to reduce the amount of blood loss during the parenchymal transection. After the operation, rats were re-warmed with an electric blanket at 36°C until

they awakened and were then returned to their cages in the laboratory.

Parenchymal transection. Occlusion of 50% of the liver mass was performed via ligation of the portal veins, which supply the RML and RL. Hepatic transection was performed by placing a clamp stepwise along the transection plane, which was marked left of the demarcation line on the median lobe following the left PVL. The middle median hepatic vein was maintained and a minimal distance of 0.5 cm was kept from the vena cava. Bleeding was prevented using a choice of compression, ligation or electrocoagulation. For the 14-day group, the ligated liver lobes (RL+RML) were excised on the 7th day after first stage surgery, and then sacrificed on the 7th days after second stage surgery (Fig. 1C and G). Daily monitoring was undertaken and body weights were recorded.

Liver acquisition and sampling. At 1 h prior to surgery, blood was collected in serum tubes (serum Z/1.2 ml; Wuxi Nest Biotechnology Co., Ltd.) via puncture of the right femoral artery and stored at -20°C until further use. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB) and total bilirubin (TBIL) levels were determined using an automated chemical analyzer (Bayer Advia 1650; Bayer AG). After the ligation of the RML and RL, ~0.5 g of liver tissue was placed in a -20°C frozen slicer (Leica CM1900; Leica Microsystems GmbH) to produce frozen sections for the early diagnosis of liver fibrosis. Hematoxylin and eosin staining were performed after embedding sections in paraffin (4 mm thick) to assess the degree of liver fibrosis for final diagnosis. Samples from group B were obtained using the same procedures. Liver lobes were weighed to calculate the liver weight/body weight ratio using the following formula: Liver weight of the individual lobe (g)/body weight (g) x100%. Furthermore, the rate of residual liver hyperplasia was used to calculate proliferation (residual liver hyperplasia rate=(postoperative liver weight-predicting preoperative liver weight)/predicted preoperative liver weight x100%). For example, the LML hyperplasia rate=(LML weight-0.004x preoperative body weight)/(0.004x preoperative body weight) x100%. Liver tissues from the RML and LML were fixed with 10% buffered formalin for 48 h at 25°C. Fibrotic and necroinflammation features were evaluated in sections (5 μm thick) stained with standard Masson's trichrome (23-25°C for 5-10 min) and hematoxylin-eosin (HE; hematoxylin, 23-25°C for 10-20 min; eosin, 23-25°C for 3-5 min), respectively, using Scheuer's scoring system (22): Stage 0, no fibrosis; stage I, expansion of the portal tracts without linkage; stage II, portal expansion with portal linkage; stage III, extensive portal to portal and focal portal to central linkage; and stage IV, cirrhosis. Other pathological features of liver tissue were evaluated using the Ishak grading system (23) and practice guidelines (24) for non-alcoholic fatty liver disease. Digital images of all slides were captured using a slide scanner (Axio Imager A2; Carl Zeiss Microscopy AG).

Immunohistochemical staining and determination of cytokine levels. The aforementioned liver specimens were dehydrated and embedded in paraffin wax (6 mm thick) for immunohistochemical staining. Primary anti-Ki-67 (cat. no. ab156956;
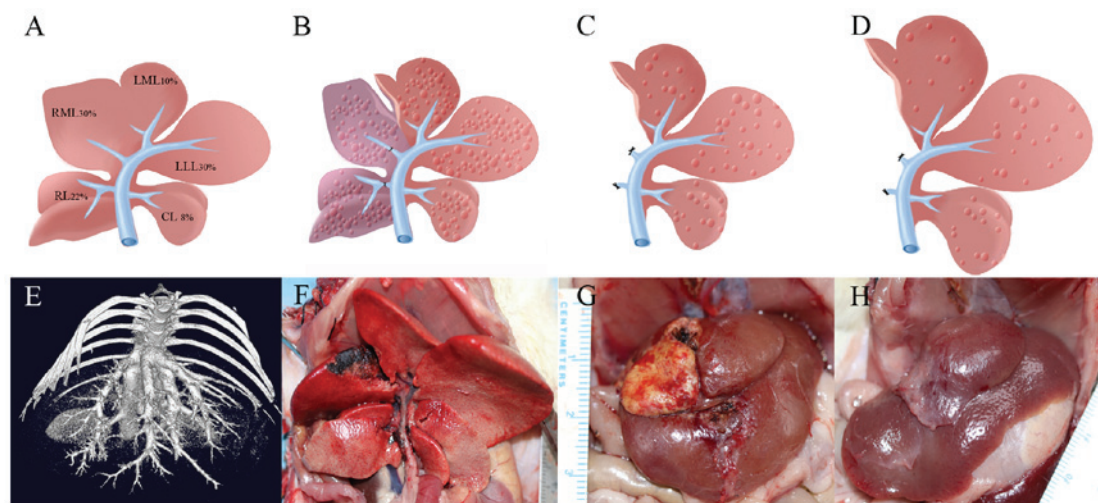
Figure 1. Construction of the ALPPS model in rats with cirrhosis. (A) A schematic of the anatomical structures in the normal rat liver and the approximate volumes of the liver lobes. (B) A schematic of liver cirrhosis after ligation of the portal veins of the RL and RML. After ligation, the liver darkened in color and the liver was separated along the middle ischemic line. (C) Day 7 after the first phase, the ligated region was excised and (D) Postoperative day 14, the remaining liver volume increased. (E) Preoperative microcomputed tomography assessment of hepatic veins and distribution characteristics. (F) The rat model of liver cirrhosis with ALPPS after surgery (after sacrifice). (G) Day 7 after step I, the ligated liver lobes appeared necrotic (yellow color). (H) Postoperative day 14, the LLL and LML had grown and the volume increased. ALPPS, Associating liver partition and portal vein ligation for staged hepatectomy; RL, right lobe; RML, right median lobe; LML, left median lobe; LLL, left lateral lobe; CL, caudal lobe.

Abcam) and anti-proliferating cell nuclear antigen (PCNA) antibodies (cat. no. ab18197; Abcam) were used to detect the respective proteins using standard immunohistochemical methods (17) in accordance with the manufacturer's protocol. The proliferation index (PI) was expressed as the fraction of proliferating hepatocyte nuclei to the total number of hepatocyte nuclei (accurate to 0.1%), as described previously (25). The numbers of Ki-67-positive and PCNA-positive hepatocytes were determined in three random visual fields under a digital slide scanner (magnification, x100; Axio Imager A2). All histological analyses were performed in a blinded fashion with respect to the experimental groups. Plasma tumor necrosis factor alpha (TNF-α; cat. no. xl-Er0359; Xinle Biological Technology Co., Ltd.), hepatocyte growth factor (HGF; cat. no. xl-Er0153; Xinle Biological Technology Co., Ltd.) and interleukin-6 (IL-6; xl-Er0196; Xinle Biological Technology Co., Ltd.) levels were determined using ELISA which was performed in accordance with the manufacturer's protocol.

Statistical analysis. Statistical analyses were performed using software packages (GraphPad Prism version 6; GraphPad Software, Inc.; SPSS 22.0 for Windows software; IBM Corp.). P<0.05 was considered to indicate a statistically significant result. The sizes of the groups (n=6) were calculated to establish a statistical power of 83.4% (G*Power; version 3.1.9.2; http://www.gpower.hhu.de/), expecting moderately high differences between medians based on previous studies of FLV hypertrophy using these techniques (17,26). All quantitative variables that were normally distributed were presented as the mean ± standard deviation and were compared using an independent Student's t-test. The median or minimum-maximum value was used to represent data that did not conform to the normal distribution. A Mann-Whitney U non-parametric test was used.

Results

Model and preoperative evaluation. In group A, 4 rats of the 36 used (11.11%; 30 were used per group, 6 additional rats used following model failure) exhibited slower absorption of modeling drugs (drug accumulation under the skin of the abdomen). Symptoms were relieved by puncturing and extruding oil. No abnormalities were observed during the modeling of group B rats. At the end of the 12th week, after an assessment of preoperative body weight between the two groups (347.56±43.22 vs. 350.81±51.22 g; P=0.45; Table I), both groups of rats were used in the experiment. Three rats of the 36 used (8.3%; 30 were used per group, 6 additional rats used following model failure) in group A exhibited moderate ascites and were therefore excluded. Furthermore, 3 other rats exhibited serious adhesions in stage II, causing excess blood loss and death. These were replaced with 6 rats with cirrhosis that were induced in the current study as aforementioned (subsequent experiments had no complications or accidents). The operations performed on rats in group B in the first and second stages were successful and no surgical incidents occurred. Postoperative recovery (determined by the regain of consciousness, limb movement and sensitivity to light) in rats with liver cirrhosis was slower than in the group B and the restoration of postoperative activity was delayed, with limb movement and normal speed returning 5-6 h after surgery. The preliminary fast paraffin sections obtained during surgery indicated that all rats in group A exhibited cirrhotic nodules and were diagnosed with cirrhosis (stage IV). Liver cirrhosis and liver fibrosis were not observed in group B.

Surgical details. The operative time for stage I was significantly longer in the group A than in group B (43.53±12.37 vs. 36.32±10.66 min; P=0.02). Intraoperative blood loss was assessed indirectly by weighing swabs. The blood loss in

Table I. Observational markers for rat ALPPS surgery.

Parameters	Group A	Group B	P-value
Number	36	30	
Preoperative body weight (g)	347.56±43.22	350.81±51.22	0.45
Modeling and surgical complications n (%)			
Slower absorption of modeling drugs	4 (11.11)	0 (0)	
Ascites	3 (8.33)	0 (0)	
Serious adhesions in stage II or death	3 (8.33)	0 (0)	
Stage I ALPPS			
Operative time (min)	43.53±12.37	36.32±10.66	0.02
Blood loss (ml)	5.33±3.09	2.36±5.33	0.04
Stage II ALPPS			
Operative time (min)	56.14±5.84	51.00±3.92	0.07
Blood loss (ml)	7.93±1.30	8.43±1.72	0.55
Rate of hyperplasia in the LML			
POD 2	48.86±0.26%	58.76±0.19%	0.004
POD 7	30.53±0.31%	22.30±0.64%	<0.001
POD 14	32.56±0.27%	33.41±0.31%	0.072
ALT POD 1 (μmol/l)	282.3-721.0	77.0-380.2	0.014
AST POD 1 (μmol/l)	219.5-1102.0	256.4-752.4	0.036
Proportion of Ki-67-positive cells POD 7	0.2616±0.0082	0.1664±0.0048	0.003
Proportion of Ki-67-positive cells POD 14	0.2804±0.0018	0.1195±0.0007	<0.001
Proportion of PCNA-positive cells POD 7	0.2151±0.0232	0.2526±0.0085	0.077
Proportion of PCNA-positive cells POD 14	0.2555±0.0155	0.1950±0.0060	0.058

Data are presented as the mean ± standard deviation or the minimum-maximum value. ALPPS, associating liver partition and portal vein ligation for staged hepatectomy; LML, left median lobe; POD, postoperative day; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PCNA, proliferating cell nuclear antigen.

stage I was 5.33±3.09 ml in group A and 2.36±5.33 ml in group B (P=0.04), indicating that greater blood loss occurred in group A than in group B. At the second stage, there was no statistical difference in operation time (P=0.07) and blood loss (P=0.55) between group A and group B (Table I).

Liver weight evaluation. In the training group, the average LML, LLL and LML liver lobe:body weight ratios were 0.004, 0.014 and 0.010, respectively (data not shown). On the third postoperative day, the body weight of group A rats decreased to the lowest value and then began to steadily increase. Group A body weights increased to a value greater than the preoperative weight by the 7th day after the operation. A similar increasing trend was observed in group B. The changes in hyperplasia were significant on the second day after surgery (Fig. 2B). On the postoperative days 2, 7 and 14, the rates of hyperplasia of the LML in the group A were 48.86±0.26, 30.53±0.31 and 32.56±0.27%, respectively. The rates of hyperplasia in the LML of group B were increased by 58.76±0.19% on day 2 (P=0.004), 22.30±0.64% on day 7 (P<0.001) and 33.41±0.31% on day 14 (P=0.072; Fig. 2B). Although a significant increase in the ratio of LLL to preoperative weight was observed in the group B, differences between the two groups were not significant (Fig. 2C). On day 3 after stage I, the weight of the atrophic hepatic lobe (RML) decreased. By the 14th day, necrosis or atrophy was gradually revealed and the value of RML/body weight decreased to the lowest point in both groups due to the lack of blood supply (Fig. 2D).

Liver function. Compared with the group B, significantly higher ALT (282.3-721.0 vs. 77.0-380.2 μmol/l; normal range, 33-98.7 μmol/l) and AST (219.5-1,102.0 vs. 256.4-752.4 μmol/l; normal range, 69.5-210 μmol/l) levels were recorded 24 h after surgery and a marked difference was observed between the two groups (P=0.014 and P=0.036, respectively, Fig. 3A and B). AST and ALT concentrations gradually returned to normal levels on postoperative day 3. However, ALB and TBIL values did not change significantly at 1, 2, 3, 7 or 14 days after surgery. (ALB normal range, 20-43 g/l; TBIL normal range, 1-20 μmol/l; Fig. 3).

Pathological changes. In the liver group A, typical areas of necrosis and hyperplasia were observed. New liver cells accumulated around the small blood vessels. As the proportion of new cells increased, changes in the fat content, ballooning and fibrosis were gradually reduced in the visual field. The proportions of Ki-67- and PCNA-positive (brown-yellow granules within the nucleus) increased after surgery (Fig. 4). At postoperative days 1, 2 and 3, cells in the group B proliferated more rapidly than in the cirrhotic group. After day 3, the
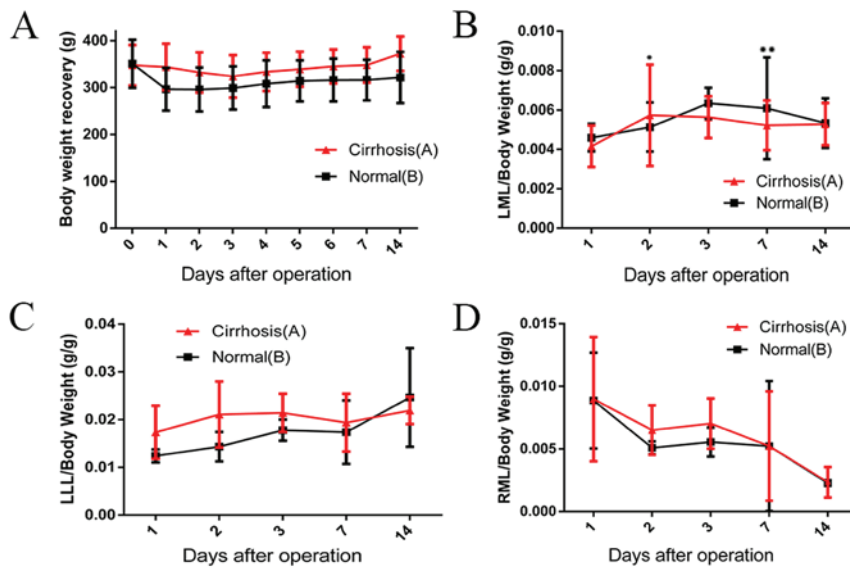
Figure 2. Postoperative weights of the body and liver lobes. (A) Postoperative weight in rats. (B) The rates of hyperplasia in the LML of the group B were increased by 58.76±0.19% on day 2 (*P=0.004), 22.30±0.64% on day 7 (**P<0.001) and 33.41±0.31% on day 14 (P=0.072). (C) The ratio of LLL to preoperative weight in the two groups was not significant. (D) Ligation of the RML hepatic lobes led to atrophy and necrosis after ischemia and the weight exhibited a substantial decrease. LML, left median lobe; LLL, left lateral lobe; RML, right median lobe.
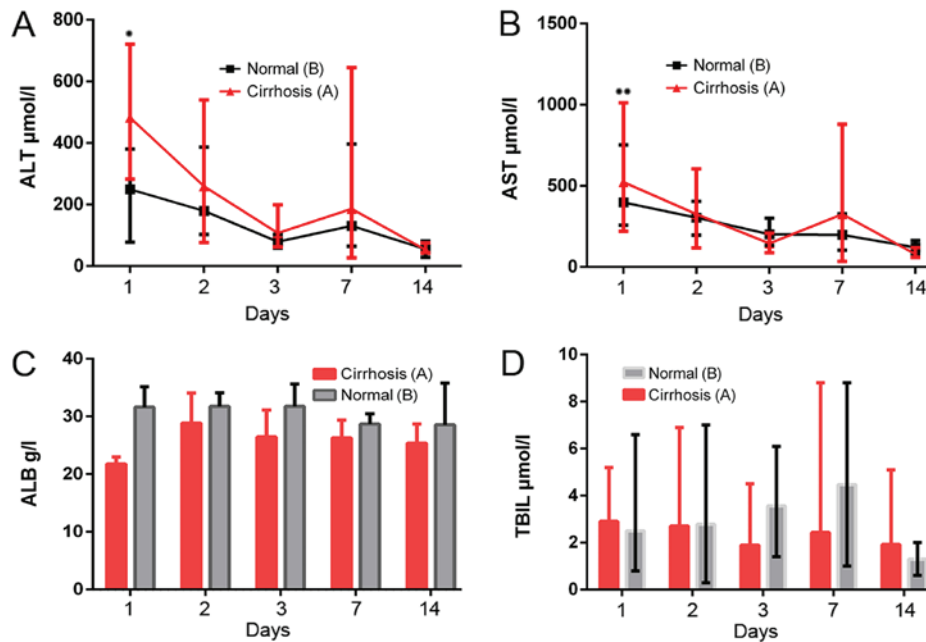

Figure 3. Postoperative liver function changes. (A) ALT and (B) AST levels increased on the day 1 post surgery (*P=0.014; **P=0.036) and then gradually decreased to a normal level on the day 3 post surgery. The postoperative effects on (C) ALB and (D) TBIL levels were smaller and were close to the reference value range. ALT, alanine aminotransferase; AST, serum aspartate aminotransferase; ALB, albumin; TBIL, total bilirubin levels.

proliferation rate in the group B began to decrease, but the rate in the cirrhotic group continued to increase slowly (Fig. 5). After surgery, the proportion of Ki-67-positive cells was significantly higher in the cirrhotic group than in the group B at 7 days (0.2616±0.0082 vs. 0.1664±0.0048, P=0.003) and 14 days (0.2804±0.0018 vs. 0.1195±0.0007; P<0.001; Fig. 5A). However, the PCNA positive cell rate did not reveal significant differences on day 7 (0.2151±0.0232 vs. 0.2526±0.0085; P=0.077) and 14 (0.2555±0.0155 vs. 0.1950±0.0060; P=0.058) after surgery between groups A and B. From the characteristics of proliferating cells (determined via Ki-67 and

PCNA-positive cells), the proliferation patterns of the group B and the cirrhotic group exhibited a trend to differ.

Serum cytokine levels. ELISA revealed different trends for different serum cytokine levels (Fig. 6). In group A, the upper limits of HGF and IL-6 levels were generally higher than in the group B and the fluctuations of the upper and lower limits were different, but the medians were similar. On the 3rd and 14th postoperative day, median HGF and IL-6 levels in group A were slightly lower than those in group B, indicating these may be important for inflammatory and proliferative responses.
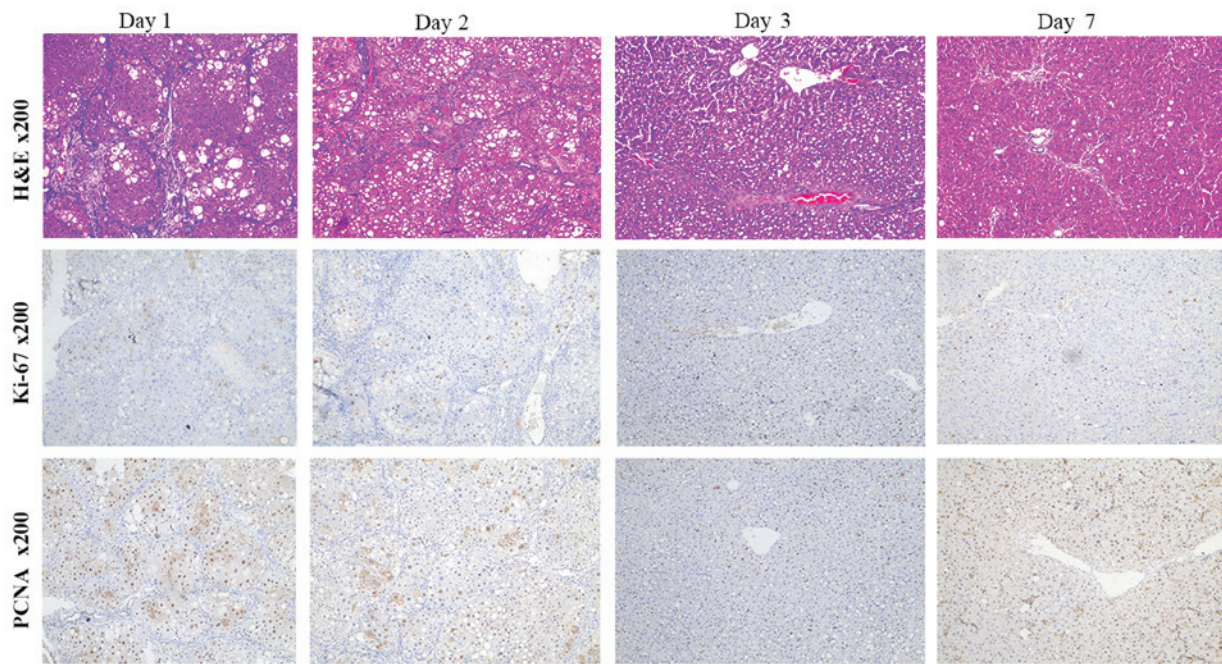
Figure 4. HE staining and characteristics of Ki-67 and PCNA staining in the cirrhotic group at 1, 2, 3 and 7 days after surgery. HE, hematoxylin-eosin; PCNA, anti-proliferating cell nuclear antigen.
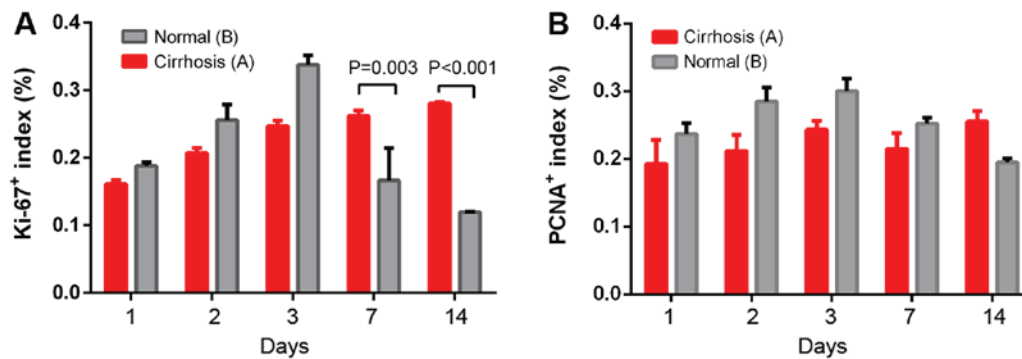

Figure 5. Ki-67-positive and PCNA-positive cell ratios after step I of ALPPS. (A) The proportion of Ki-67 positive cells in group B peaked on postoperative day 3 and then decreased. In group A, the growth was continuous and slower, surpassing group B on day 7 (26.16±0.82 vs. 16.64±4.76%; P=0.003) and hyperplasia persisted 14 days after surgery. (B) The trend for PCNA-positive cells in group B was consistent with the trend for Ki-67-positive cells, but trends did not exhibit statistical significance. PCNA, anti-proliferating cell nuclear antigen.

The individual differences were larger; while the levels in group B exhibited a more gradual decrease. Despite the value of TNF-α being higher than that of group B on the 1st day after surgery, the overall trend of each group was downward and no statistical difference was identified. Significant differences in the changes in cytokine levels were not observed between the two groups on postoperative days 1, 2, 3, 7 and 14 (Fig. 6).

Discussion

The clinical application of ALPPS in advanced primary and metastatic liver cancer is an innovative and important, yet challenging surgical strategy (10,27). However, complications and mortality in the perioperative period remain high and the efficacy of this procedure in oncology is inconclusive (28). Currently, ALPPS is used in cases of liver metastasis in colon cancer and Barcelona stage C liver cancer (28,29). Various

scholars (9,10,30,31) are cautious about using ALPPS to treat advanced HCC due to faster progression, increased liver fibrosis, increased cirrhosis, insufficient residual hepatic hyperplasia and postoperative liver failure. A large proportion of newly diagnosed middle- and late-stage liver cancer cases in China each year are accompanied by varying degrees of cirrhosis (12,32). Many patients are unable to undergo surgery due to large tumors. Palliative surgery including PVL and PVE, is often used as treatment for HCC which can reduce the size of the tumor, delay tumor progression and create conditions and opportunities for treatment such as ALPPS (33). ALPPS represents a lifeline for patients with large tumors and hepatocellular carcinoma with multiple masses. R0 [no residual tumor under the naked eye or under the microscope (negative margin)] removal can cause many problems and the mechanism of postoperative residual liver regeneration is unclear, particularly for livers with hepatitis B after liver
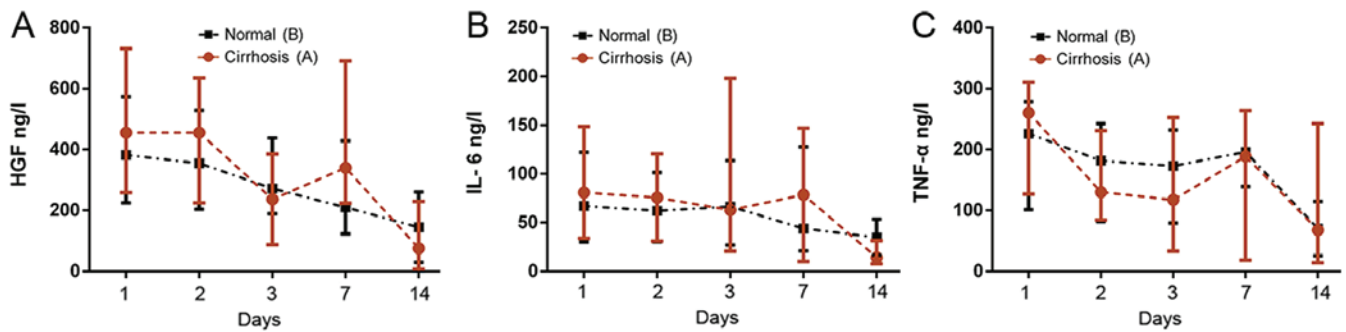
Figure 6. Detection of (A) HGF, (B) IL-6 and (C) TNF-α levels after surgery using ELISA in the two groups. Significant differences in the changes in cytokine levels were not observed between the two groups on postoperative days 1, 2, 3, 7 and 14. HGF, hepatocyte growth factor; IL-6, interleukin-6; TNF-α, plasma tumor necrosis factor alpha.

cirrhosis (32). A current topic of academic debate is whether the liver is healthy during short-term regeneration (10,31,34). The present study therefore, established an ALPPS model in cirrhotic rats to further explore this phenomenon.

An ALPPS model was established by ligating the portal veins of the RL and RML. The RML, CL and LLL account for ~50% of the total liver volume and were therefore used as the remaining liver in the cirrhotic rats. This ligation method was used to avoid serious complications caused by wounds and to ensure surgery was as safe as possible. Due to preliminary experimental results, the study was unable to mimic 70% of the liver volume in cirrhotic rats (data not shown). This smaller future liver remnant (≤30%) may have influenced the outcome of the current study, however this requires further study. ALPPS surgery in the context of cirrhosis still maintained significant proliferative capacity and facilitated second stage hepatectomy in a short period of time. On a normal liver background, days 1 and 3 after ALPPS constitute the critical period for liver regeneration, the rate of liver regeneration slows after 7 days (17,26,33,35). However, in the present study, slow hyperplasia was observed in the liver with cirrhosis. Ki-67 and PCNA-positive cells continued to progress through the cell cycle at days 7 and 14 with no significant peaks. This outcome however, were not the same as the proliferation curve in the group B and the pattern of proliferation differed in cirrhotic livers. Liver regeneration after ALPPS in rats with liver cirrhosis is slower than in a normal liver, likely due to an intrahepatic portosystemic shunt blockage after hepatic parenchymal disconnection (32). The results of the current study indicated that the regeneration rate in cirrhotic rats started later but continued for longer than in normal rats. The remaining liver has a more abundant portal vein blood supply and aggravates the pressure of the remaining hepatic portal vein, causing it to harden. In addition, the liver nodules are affected by blood flow (33). In the current study, the observation endpoint was 14 days after surgery. This occurred as weights exceeded those of the preoperative stage 14 days after surgery, indicating that the remaining liver function may have returned to normal. It is hypothesized that after 14 days, the degree of cirrhosis may be slightly reduced and the proportion of regenerating liver tissue may gradually increase after ALPPS in the rat model of cirrhosis. However, how this new liver tissue breaks through the liver fiber structure requires further research. Currently,

the present study have two hypotheses: One is that the fiber strands are pushed to one side and the new cells break through the narrow gap to grow; the other is that the newborn cells or mesenchymal cells secrete cytokines to induce the dissolution of fiber strips and various molecules cause cracking and breakage of the fiber strands, giving the newborn cells enough space to grow. Future experiments will aim to validate these hypotheses.

Schlegel et al (19) established an ALPPS model in mice with normal livers to determine the mechanism by which ALPPS promotes liver regeneration. The FLV growth rate of the ALPPS group was twice the rate of the PVL group and higher levels of IL-6 and PCNA were detected than in the controls (29). In the PVL group, additional injury to other organs (radiofrequency ablation of the spleen, kidney or lung) was performed and plasma was then harvested and injected into the PVL group. Finally, the authors observed a similar increase in FLV to the ALPPS group, indicating that localized trauma or inflammatory responses might accelerate the induction of hepatocyte proliferation (19). Almau et al (36) and Tong et al (37) established a rat experimental model based on the ALPPS procedure as described above. Furthermore, Yao et al (38) revealed that the reactivation rate of the liver was significantly faster in the ALPPS group on the 3rd and 7th postoperative days compared with the PVL group. The mechanism of ALPPS proliferation that was reported includes massive tissue necrosis and inflammatory responses after liver disconnection. This stimulates liver regeneration and upregulation of cytokine expression in regenerated lobes. However, the ALPPS models reported in the aforementioned studies were all based on a normal liver background and did not simulate liver regeneration in the cirrhotic liver. The current study emphasizes the safety and proliferative capacity of ALPPS after cirrhosis (stage IV). Compared with liver fibrosis (not all F4 grade), animal models of cirrhosis are slightly more difficult to establish (39).

Currently, the methods for clinically assessing the regeneration of remaining liver tissue depends on CT liver volume reconstruction, but liver hyperplasia is roughly estimated and may differ from the actual area (40). Therefore, doctors suspect that the proliferation of the remaining liver volume is due to the regeneration of liver cells or hepatocyte parenchyma edema (34). The current study measured the weight of each lobe of the liver on postoperative days 1, 3,

5, 7 and 14 to calculate the remaining liver growth rate. The present study investigated Ki-67-positive and PCNA-positive proliferating cells using immunohistochemistry to measure the number of cells in the regenerating liver that were undergoing cell proliferation at different time points. The results indicate that proliferation accompanied by cell division and the number of cells was significantly increased. However, whether hepatocyte proliferation is derived from hepatic stem cells or bile duct-derived cells, needs further investigation. ALPPS represents one step beyond PVL or PVE but significantly balances time between liver regeneration and tumor recurrence. The mechanism by which ALPPS promotes liver regeneration is not yet clear. Previous studies have indicated that it may be associated with changes in hepatic blood flow caused by the portal veins of the ligated hepatic lobe (41), the inflammatory response and stress response caused by disassociation of the liver or changes in proliferation-associated factors (17,42).

In the current study, serum levels of TNF-α, IL-6 and HGF were measured to further explore the molecular mechanisms. Compared with the normal liver group, TNF-α and IL-6 levels in the 1st, 2nd, 7th days after surgery were elevated in the ALPPS group with cirrhosis. TNF-α and IL-6 serve important roles in the initial stages of liver regeneration (40). These two pro-inflammatory cytokines are produced by activated Kupffer cells in the liver and promote the transition of hepatocytes from the G0 phase to the G1 phase of the cell cycle (43). Hepatocytes are sensitive to growth factors such as HGF and synthesize DNA (44). Therefore, ALPPS may promote the onset of liver regeneration by upregulating these two pro-inflammatory cytokines. The group A exhibited elevated cytokine levels, particularly of HGF and IL-6. The present study indicated that increased cytokine levels detected in the current experiments may be associated with pro-inflammatory and stress responses induced by cirrhosis itself. This cirrhosis may be caused by surgical trauma and necrosis of the hepatocytes due to decreased microcirculation in the LML (44). The results indicate that an inflammatory response is inhibited in the presence of cirrhosis, but more samples are required to confirm this hypothesis.

In the current study, the effects of ALPPS on the FLV-induced rapid liver growth were assessed by the comparison of cirrhotic and normal liver tissues. FLV growth, liver function changes and postoperative serum cytokine levels were analyzed. As the first cirrhosis animal model of ALPPS, the preliminary results of the current study validated the safety and proliferative capacity of the surgery. The source of new liver tissue and how the new cells break through the fiber structure will be studied in further investigations. The biological behaviors and liver structure in the human body are more complex than in tumor-free rats with liver cirrhosis. Therefore, the two models cannot be accurately compared. Furthermore, when combined with liver cancer, liver cirrhosis in the context of mechanisms to promote liver regeneration requires further research.

Availability of data and materials

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

Authors' contributions

WW proposed the study. XY and CY performed the experiments and wrote the first draft. XY collected and analyzed the data. All authors contributed to the design and interpretation of the study and to further drafts.

Ethical approval and consent to participate

All procedures were performed according to the guidelines and with the approval of the Animal Care and Ethics Committee of the West China Hospital of Sichuan University (Permission no: 2017001A).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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