Bile Acid Overload Induced by Bile Duct and Portal Vein Ligation Improves Survival after Staged Hepatectomy in Rats*

Xin-lan GE[1, 2, 3†], Xuan ZHANG[1, 2, 3†], Chong-hui LI[1, 2, 3], Ke PAN[1, 2, 3], Lei HE[1, 2, 3#], Wei-zheng REN[1, 2, 3#]
[1]*Faculty of Hepato-Pancreato-Biliary Surgery, First Medical Center, Chinese PLA General Hospital, Beijing 100853, China*
[2]*Institute of Hepatobiliary Surgery of Chinese PLA, Beijing100853, China*
[3]*Key Laboratory of Digital Hepatobiliary Surgery, PLA, Beijing 100853, China*

[Abstract] Objective: Compared to portal vein ligation (PVL), simultaneous bile duct and portal vein ligation (BPL) can significantly enhance hypertrophy of the intact liver. This study aimed to investigate whether BPL could improve survival after extended hepatectomy independently of an increased remnant liver. **Methods:** We adopted rat models of 90% BPL or 90% PVL. To investigate the role of bile acids (BAs), the BA pools in the PVL and BPL groups were altered by the diet. Staged resection preserving 10% of the estimated liver weight was performed 3 days after BPL, PVL, or sham operation. Histology, canalicular network (CN) continuity, and hepatocyte polarity were evaluated. **Results:** At 3 days after BPL, PVL, or sham operation, when the volumetric difference of the intended liver remained insignificant, the survival rates after extended hepatectomy were 86.7%, 47%, and 23.3%, respectively (P<0.01). BPL induced faster restoration of canalicular integrity along with an intensive but transient BA overload. Staged hepatectomy after BPL shortened the duration of the bile CN disturbance and limited BA retention. Decreasing the BA pools in the rats that underwent BPL could compromise these effects, whereas increasing the BA pools of rats that underwent PVL could induce similar effects. The changes in CN restoration were associated with activation of LKB1. **Conclusion:** In addition to increasing the future remnant liver, BPL shortened the duration of the spatial disturbance of the CN and could significantly improve the tolerance of the hypertrophied liver to staged resection. BPL may be a safe and efficient future option for patients with an insufficient remnant liver.

Key words: bile canalicular network; hepatocyte polarization; liver regeneration; portal vein ligation; simultaneous bile duct and portal vein ligation

Liver resection is a procedure widely adopted as the first and sometimes the only curative choice for patients with various liver diseases[1]. However, an insufficient future remnant liver (FRL) too often renders such a procedure infeasible[1–3]. Due to its ability to induce hypertrophy of the intended liver, portal vein ligation (PVL) has been applied routinely in case of an insufficient FRL[4, 5]. Staged hepatectomy can be performed with improved safety when the hypertrophied intact liver is sufficient in volume. Unfortunately, this procedure requires prolonged waiting and is frequently associated with a poor response[4, 6]. Therefore, improved methods are urgently needed.

Clinically, it has been demonstrated that when diseases cause obstructed branches of the bile duct system, the intact liver exhibits hypertrophy[7–9]. Previously, we have found that bile duct and portal vein ligation (BPL) can further enhance hypertrophy of the intact liver, resulting in a significantly larger FRL than PVL[7]. Surprisingly, our unpublished data show that even at a very early stage, when the volumetric difference of the FRL remains insignificant, the survival rate after staged hepatectomy is also remarkably improved in rats that received 90% BPL than those undergoing 90% PVL. This incidental finding strongly suggests that in addition to increasing the FRL volume, BPL seems to improve the functional reserve of the hypertrophied FRL.

Hepatocytes, like other epithelia, are highly

Xin-lan GE, E-mail: gexinlan@301hospital.com.cn; Xuan ZHANG, E-mail: zhangxuandebox@163.com
†The authors contributed equally to this work.
#Corresponding authors, Wei-zheng REN, E-mail: rwz301@163.com; Lei HE, E-mail: bud-lotus@sohu.com
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polarized cells with distinct apical and basal domains. Bile canaliculi, the smallest branches of the biliary system, are formed by the apical domains of adjacent hepatocytes[10]. The canaliculi are continuous, interconnecting each hepatocyte couplet and constructing a network throughout the hepatic lobule. Bile secreted by hepatocytes is delivered through this network. Therefore, a well-branched canalicular network (CN) is the underpinning of normal bile secretion and drainage[10]. Rapid hepatocyte proliferation after hepatectomy results in poorly polarized neogenetic hepatocytes[10–12] and a disrupted CN[13–16]. Prolonged disturbance of the CN is strongly associated with liver failure after hepatectomy, transplantation, or hepatic injury[3, 16–19]. Recently, bile acids (BAs) have attracted much attention as extrinsic polarization cues for hepatocytes, promoting the formation and/or re-establishment of the CN[1, 2, 10, 18–20]. Fu *et al* have described a unique function of taurocholate in canaliculi formation involving signaling through a cAMP-Epac-MEK-Rap1-LKB1-AMPK pathway[19, 21]. In addition, Ikebuchi *et al* have shown that ursodeoxycholic acid stimulates the formation of the CN *in vitro* and *in vivo*[18]. Our previous study also revealed an enhanced BA overload in the intact liver after BPL[7], which might lead to faster CN reconstruction and pre-activation of the polarity-establishing signaling pathway, contributing to the improved prognosis of patients.

Considering these facts, we hypothesized that the enhanced BA overload after BPL accelerates hepatocyte polarization and CN reconstruction, which are responsible for the significant improvements in the functional reserve of the FRL. To test our hypothesis, we established rat models of BPL and PVL and compared their effects on hepatocyte polarization and CN reconstruction as well as the tolerance of extended hepatectomy with a similar FRL. Furthermore, the role of BAs in accelerating CN reconstruction was examined more directly in rats whose BA pools were altered when they received PVL or BPL.

1 MATERIALS AND METHODS

1.1 Animal Models

We used 7-week-old male Sprague-Dawley (SD) rats weighing 220–250 g (Laboratory Animal Research Center of the Academy of Military Medical Science, China) for all models. The Institutional Animal Care and Use Committee of the Chinese PLA General Hospital approved all experimental protocols. The procedures were performed as described in the supplementary materials and methods section and illustrated in fig. S1. In our previous report, we found that the posterior caudate lobe accounted for approximately 10% of the estimated liver in the BPL or PVL groups on postoperative day 3, while the whole caudate lobe accounted for approximately 10% of the liver in the sham group[7]. For staged resection, all rats received 90% hepatectomy on day 3 after the primary procedure. In particular, a posterior-caudate-lobe-sparing hepatectomy was performed after 90% PVL (V-Hx) or 90% BPL (B-Hx), while a hepatectomy preserving the whole caudate lobe on day 3 after the sham operation (S-Hx) was performed (fig. 1A). To alter the BA pools, a diet supplemented with taurocholate (0.2%, Sigma-Aldrich, USA) was fed to the rats that underwent PVL (tPVL), and a diet supplemented with cholestyramine (2%, Sigma-Aldrich, USA) was fed to the rats that received BPL (rBPL), starting from 1 day before the procedure until the animals were sacrificed or received a staged resection. After staged hepatectomy, the diet shifted to a regular diet immediately after the resection; tPVL followed by resection was indicated as tV-Hx, and rBPL resection as rB-Hx (fig. 1B). The rats were sacrificed as indicated (fig. 1A and 1B). In each group, 30 rats were used for the survival analysis.

1.2 Hepatic BA, Liver Weight, and Serum Tests

Extraction of hepatic BAs was performed as described previously[7, 22]. The rats and livers were weighed, and liver weight was calculated as the percentage of the estimated liver weight. The serum markers and hepatic BA levels were measured using a serum analyzer (Cobas-Mira Plus; Roche, Switzerland).

1.3 Histology, Immunofluorescence, and Immunohistochemical Staining

Suzuki's liver histological damage scoring system was used to evaluate the liver samples as follows: 0, no necrosis; 1, single cell necrosis; 2, <30% necrosis; 3, 30%–60% necrosis; 4, >60% necrosis; 0, no vacuolization; 1, minimal vacuolization; 2, mild vacuolization; 3, moderate vacuolization; 4, severe vacuolization[23]. For each animal, 20 random visual fields (400×) were scored. Bile canaliculi were visualized by ABCB-1 staining, and the slides were first incubated with mouse anti-ABCB1 antibody (1:30, Enzo Lifesciences, USA), washed with PBS, then incubated with goat anti-mouse IgG H&L (Alexa Fluor® 488, 1:500; Invitrogen, USA). DAPI (Sigma-Aldrich, USA) was used to visualize the nuclei. An Olympus Fluoview FV1000 confocal laser scanning microscope (Japan) was used to obtain images. Specimens were scored for ABCB1 continuity in 20 random high-power fields (400×). The continuity scoring system was as follows: 0, no staining; 1, spotty appearance; 2, interconnecting a few hepatocytes; 3, interconnecting more than 4 hepatocytes; 4, interconnecting more than 8 hepatocytes. The Image J program (National Institutes of Health, USA) was used to measure the length of the canaliculi within the image field. Liver samples were also stained with purified mouse anti-human Ki-67 (1:50, BD

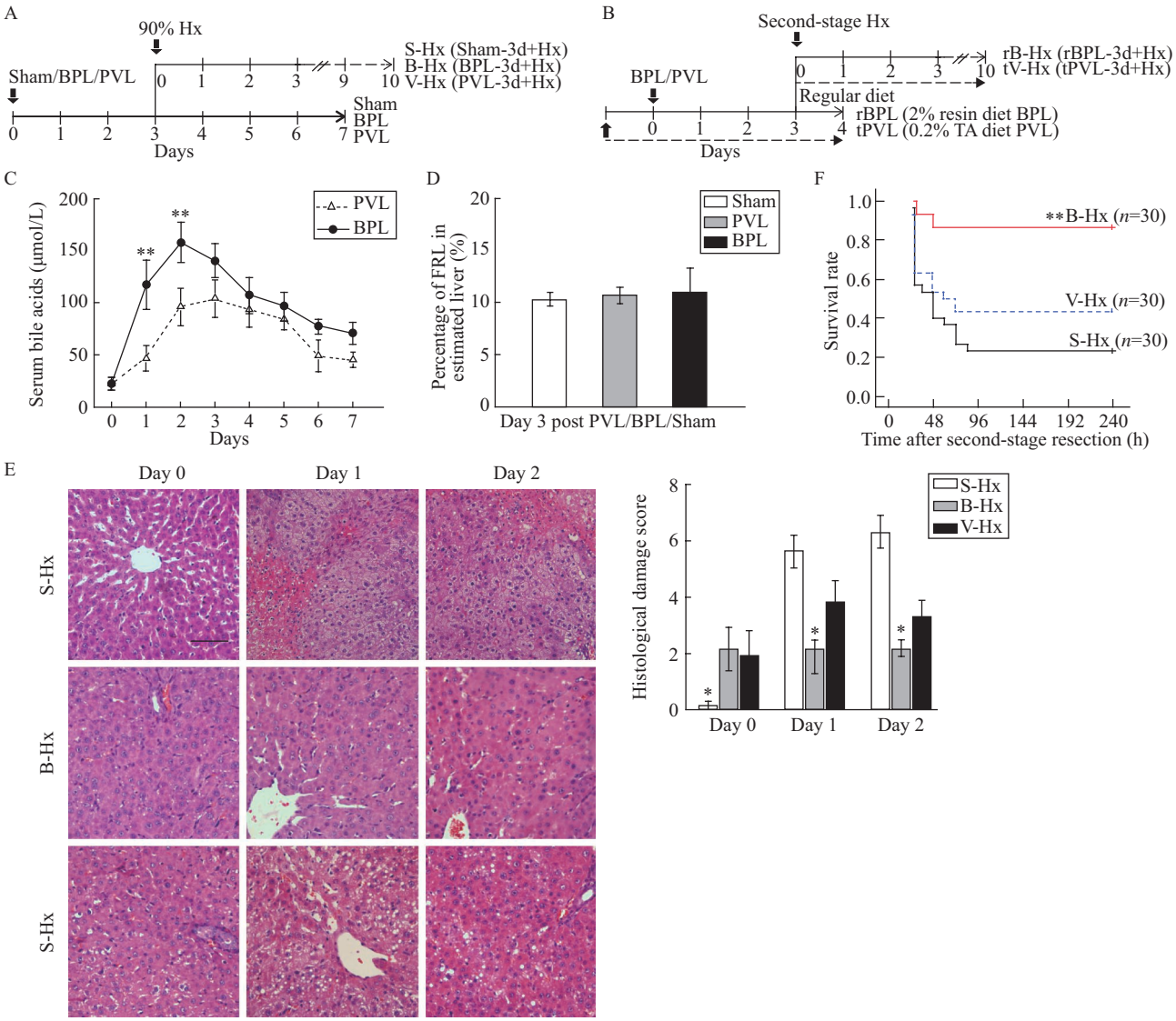

Fig. 1 A: Experimental design. The rats were randomized into the sham, BPL, and PVL groups. Staged hepatectomy was performed on day 3 after the sham operation (S-Hx), BPL (B-Hx), and PVL (V-Hx), respectively. B: Experimental design. A diet supplemented with 0.2% taurocholate (TA) was fed to rats that underwent PVL (0.2% taurocholate, tPVL), and 2% cholestyramine (resin) was fed to rats that underwent BPL (2% resin, rBPL), since 1 day before the procedure until sacrifice or staged hepatectomy. After staged hepatectomy on day 3 post tPVL (tV-Hx) or rBPL (rB-Hx), the rats shifted back to a regular diet. C: Serum bile acid levels after BPL or PVL. D: On day 3 after BPL, PVL, or sham operation, FRL accounted for approximately 10% of the estimated liver weight in all three groups (P>0.10). E: Representative images of liver sections after staged resection, and the histological damage was scored as described. F: Survival after staged hepatectomy. Scale bar = 50 μm. The data are presented as mean ± SD, $^{*}P$<0.05, $^{**}P$<0.01 *vs*. S-Hx group

Pharmingen, USA), and the Polink-2 HRP Plus Mouse DAB Detection System for immunohistochemistry was used (Golden Bridge International, USA). The Ki-67 labeling indexes were calculated by determining the number of Ki-67-positive hepatocyte nuclei/total hepatocyte nuclei in 20 random visual fields (400×).

1.4 Western Blot Analysis

Western blotting was performed as described previously. In brief, the PVDF blots were obtained after SDS/PAGE, transfer, and blocking, then incubated with the following primary antibodies: rabbit anti-LKB1, anti-AMPK, anti-phospho-AMPK (Thr-172), anti-phospho-MEK1/2, mouse anti-MEK1/2 (Ser217/221) antibody (1:1000, Cell Signaling Technology, USA), rabbit anti-phospho-LKB1 (Ser-431), and mouse anti-GAPDH antibody (1:5000, Sangene Biotech Co., China). Secondary antibodies from Santa Cruz Biotechnology (USA) were used. The membranes were finally developed with a Super-Signal chemiluminescent substrate. Image J software was used to measure the band densities.

1.5 Statistical Analysis

All variables are presented as the mean ± SD. All statistical analyses were performed using SPSS, version 27.0 (SPSS Inc, USA). The Student's *t*-test, Kaplan-Meier method, rank-sum test, or one-way ANOVA were used as appropriate. *P*-values less than 0.05 were considered significant.

2 RESULTS

2.1 BPL Improves Survival after Extended Hepatectomy Independently of Increased FRL

We adopted the rat model of 90% BPL and 90% PVL, as established previously by us (fig. S1A)[7]. Consistent with our previous report, all the rats tolerated the procedures well. In both groups, HE staining showed limited histological damage in the hypertrophied and ligated lobes (P>0.10, fig. S1B). Cholangiocyte proliferation was again evident in the ligated liver after BPL. Compared to PVL, the serum BA levels were significantly increased on day 1 and day 2 after BPL (fig. 1C).

In the BPL, PVL, and sham groups, 6 rats were sacrificed on day 3 to estimate FRL. We found that on day 3 post ligation/sham, the FRL accounted for approximately 10% of the estimated liver in the BPL, PVL, and sham groups (P>0.1, fig. 1D). Staged resection was therefore performed on day 3 after PVL (V-Hx), BPL (B-Hx), or sham operation (S-Hx, fig. S1A) to evaluate the tolerance of the FRL to extended hepatectomy. The survival rates were 23.3% in the S-Hx group, 47% in the V-Hx group, and 86.7% in the B-Hx group (P<0.01; fig. 1F). In the S-Hx group, consistent with previous reports, the liver damage markers and total bilirubin were drastically elevated in the surviving rats, and there was severe vacuolization and patchy necrosis in the remnant liver, indicating hepatic insufficiency as a major cause of mortality[24]. The serum markers were decreased and histological damage was mitigated in the V-Hx group. As anticipated, the rats in the B-Hx group showed the lowest levels of liver damage markers, total bilirubin, and serum BA among the three groups (fig. S1C–S1F) as well as the lowest histological damage scores on postoperative day 1 and day 2 (fig. 1F).

According to the Ki67 labeling indexes, the S-Hx group showed a delayed initiation of mitosis followed by dramatic elevation of Ki67 labeling indexes, indicating a poor regenerative response. While in the other groups, the indexes were constantly high with no significant difference between the B-Hx and V-Hx groups (fig. S1G). With the same FRL, BPL significantly improved survival after staged extended resection, indicating an effect independent of an increased FRL.

2.2 BPL Induced Faster Restoration of Canalicular Integrity Along with a More Intensive but Transient BA Overload

In the normal liver, the expression of ABCB1 represents the bile canaliculi. Therefore, ABCB1 staining is usually used to visualize bile canalicular integrity[19, 21, 25–27]. In the sham group, ABCB1 staining was localized in typical bile canaliculi and appeared to interconnect each cell, constructing networks throughout the lobule (fig. S2A). After PVL or BPL, sequential destruction and reconstruction of the bile CN was observed (fig. 1A). The average length of canaliculi dropped markedly during the first 2 days. However, on day 3, an earlier recovery of the canaliculi length was observed in the BPL group. In contrast, the bile CN of the PVL group progressively deteriorated on day 3. The changes in the bile CN continuity were comparable to those of the canaliculi length.

Compared to PVL, a transient but more intensive hepatic BA overload was detected after BPL (fig. 2B)[7]. BPL moderately intensified hepatic BA retention, followed by an earlier and more abrupt decrease, consistent with the restoration of the bile CN. These findings indicate an earlier compensation of bile drainage.

2.3 Staged Hepatectomy after BPL Showed a Shorter Duration of Bile CN Disturbance and Limited BA Retention

After staged resection, the B-Hx group constantly manifested a superior average length and canalicular continuity during the first 2 postoperative days, which were significantly greater than those in the V-Hx and S-Hx groups (fig. 2C). By day 3, a well-branched CN was detected in almost all of the surviving animals.

Despite the highest hepatic BA concentration at the time of resection, B-Hx constantly manifested the least amount of hepatic BA after staged resection (fig. 2D).

2.4 BA Feeding to PVL Rats Improved Tolerance to Extended Hepatectomy and Bile Canalicular Integrity

In an attempt to replicate the BA overload induced by BPL, we fed the PVL rats a diet supplemented with 0.2% taurocholate (tPVL group). The diet started from 1 day before PVL and continued until the staged hepatectomy or sacrifice. More prompt restoration of the canalicular integrity along with markedly intensified serum and intrahepatic BA retention were observed (fig. 3A and 3B). Primary recovery of the branched bile CN appeared as early as 2 days after ligation, followed by continuous elongation and interconnection; it was constantly superior to those of the PVL group (fig. 3A). The same staged hepatectomy procedure was performed on the day 3 after PVL, with the diet switching back to a regular diet postoperatively. We found that compared to the V-Hx group, the survival rate was significantly increased in the tV-Hx group (70.0% *vs*. 47.0%, P<0.05, fig. 3E). Meanwhile, we also observed decreases in AST, ALT, and total bilirubin levels in the B-Hx group compared to the V-Hx group, although the differences were not significant (fig. S2B–S2E).

2.5 BA Depletion in BPL Rats Decreased Tolerance to Extended Hepatectomy and Aggravated BCN Disturbance

To further elucidate the role of BA in ameliorating

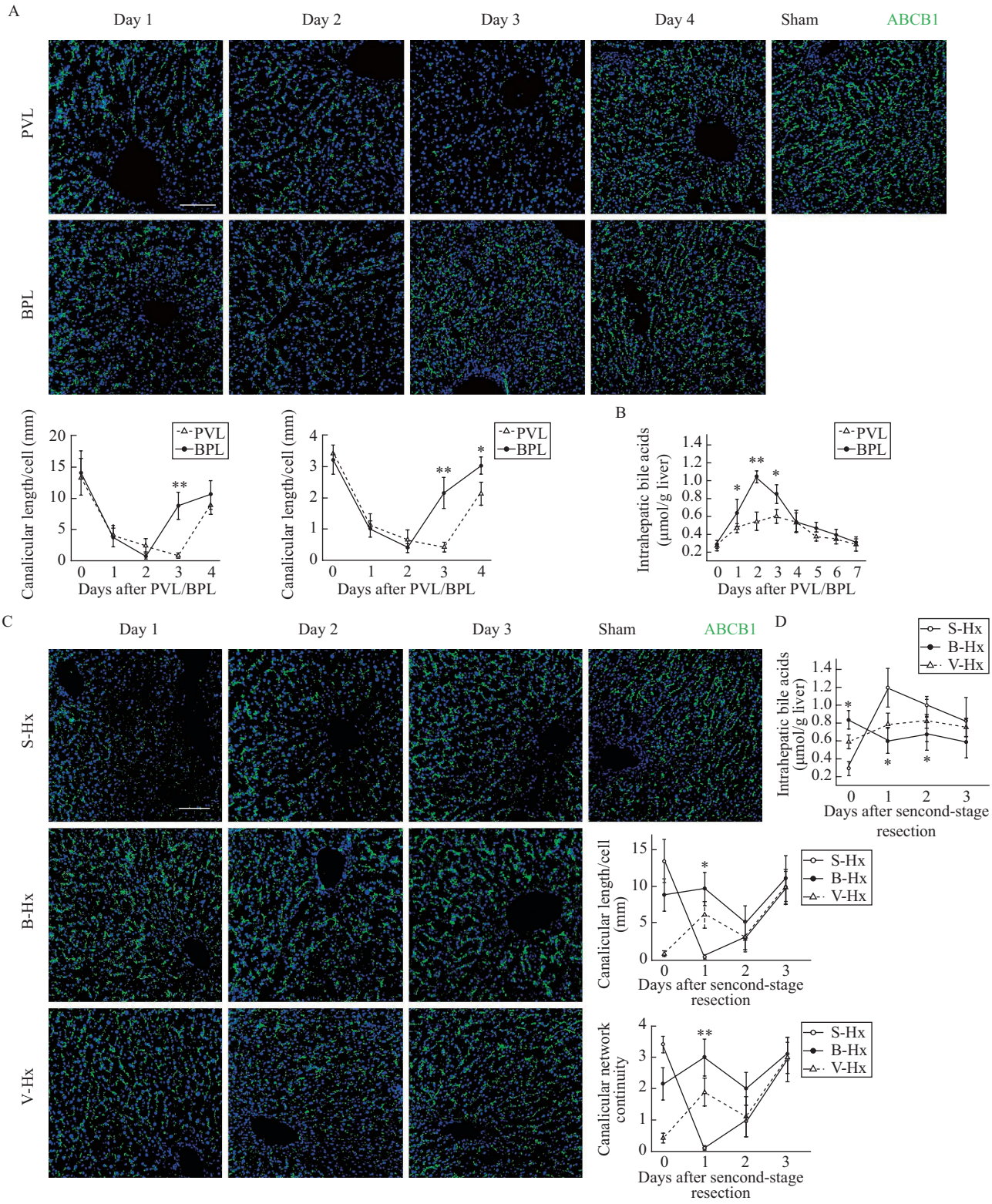

Fig. 2 A: Daily progress of disturbance of the canalicular network and reconstruction in the hypertrophied liver after BPL or PVL was visualized by staining of ABCB1, with the average canalicular length per cell and continuity evaluated. B: Hepatic bile acids levels in the intact liver after BPL or PVL. C: Canalicular network after staged resection, with the average length per cell and continuity assessed. D: Changes of hepatic bile acids in the liver remnant after staged resection. Scale bar = 100 μm. The data are presented as mean ± SD, $^{*}P<0.05$, $^{**}P<0.01$ *vs*. S-Hx group

the functional reserve of FRL, we depleted the endogenous BA by feeding a diet supplemented with 2% cholestyramine to the BPL rats (rBPL group). The diet started from 1 day before BPL and continued until the staged hepatectomy or sacrifice. After cholestyramine feeding, prolonged canalicular disturbance along with much lower serum and intrahepatic BA retention were observed (fig. 3C and 3D). Even on day 4 after BPL, the

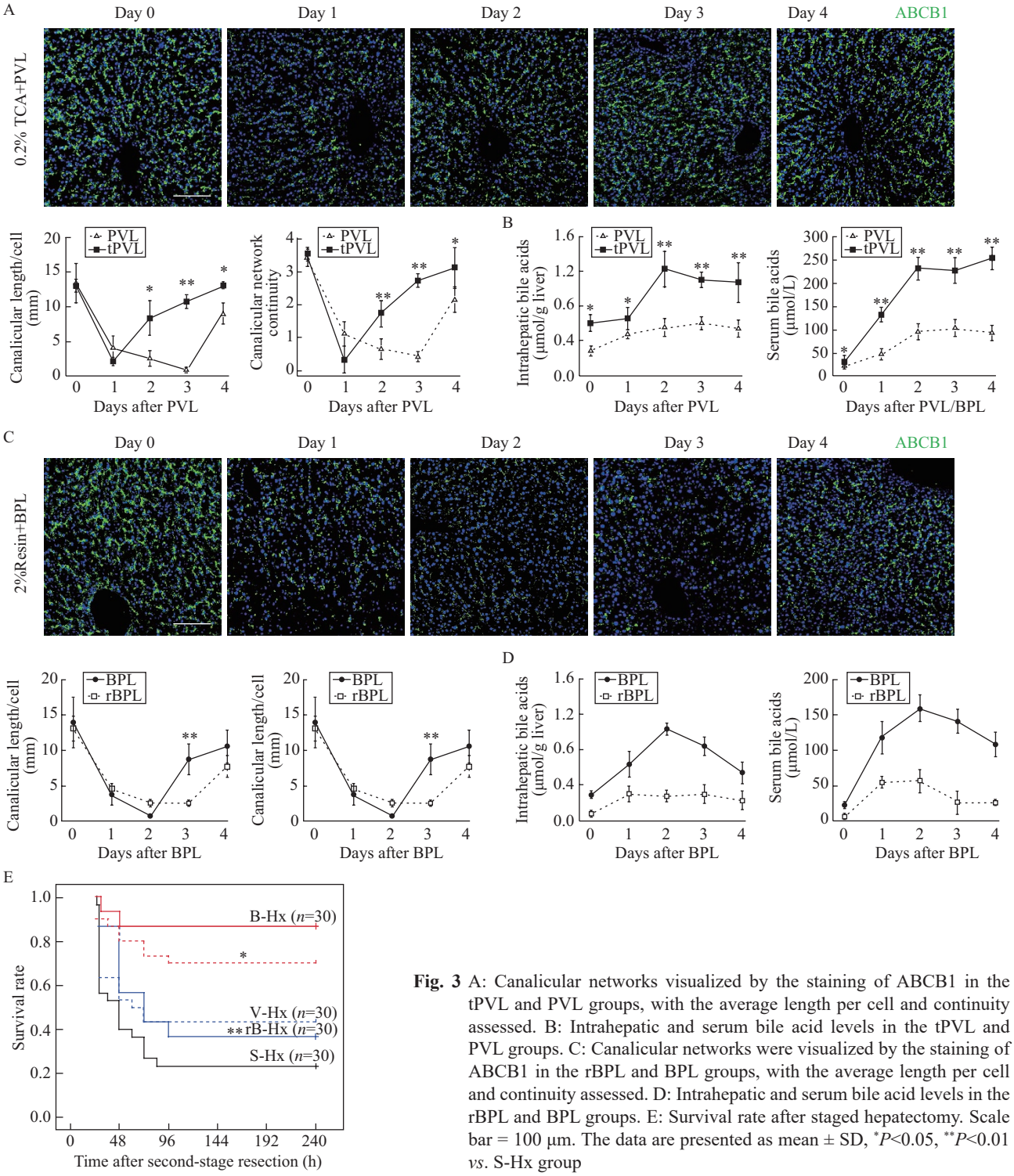

Fig. 3 A: Canalicular networks visualized by the staining of ABCB1 in the tPVL and PVL groups, with the average length per cell and continuity assessed. B: Intrahepatic and serum bile acid levels in the tPVL and PVL groups. C: Canalicular networks were visualized by the staining of ABCB1 in the rBPL and BPL groups, with the average length per cell and continuity assessed. D: Intrahepatic and serum bile acid levels in the rBPL and BPL groups. E: Survival rate after staged hepatectomy. Scale bar = 100 μm. The data are presented as mean ± SD, $^{*}P<0.05$, $^{**}P<0.01$ *vs*. S-Hx group

bile CN remained disturbed without evident restoration and was constantly inferior to those of the rBPL group. The same staged resection was performed on day 3 after BPL, with the diet switching back to a regular diet postoperatively. We found that compared to the B-Hx group, the survival rate of the rB-Hx group after the staged hepatectomy was dramatically decreased (36.7% *vs*. 86.7%, $P<0.01$, fig. 3E). Consistently, the serum liver damage markers AST, ALT, and total bilirubin were higher than those of the B-Hx group, although the differences were not significant (fig. S2B–S2E).

2.6 The Change in CN Restoration before and after Staged Hepatectomy Is Associated with LKB1

The polarity of hepatocytes underlies the structure of the bile canaliculus. BAs can stimulate hepatocyte polarization through a cAMP-Epac-MEK-LKB1-AMPK pathway[10, 19, 21, 25]. To further investigate the molecular mechanism by which BAs accelerate bile canalicular formation, the protein levels of several key

molecules, including MEK1/2, LKB1, and AMPK, were analyzed in the hypertrophied lobes. In the BPL group, the activation of LKB1 as indicated by the level of phosphorylated LKB1 (P-LKB1) was upregulated sharply and was significantly greater than that in the PVL group during the first 3 days after BPL or PVL (fig. 4A).

After staged resection, the activation of this polarization-associated signaling pathway was most prominent and swift in the B-Hx group, minor in the V-Hx group, and markedly delayed and compromised in the S-Hx group (fig. 4B).

The expression levels of the MEK-LKB1-AMPK signaling pathway were also investigated in the BA feeding (tPVL) and depletion (rBPL) groups. It was obvious that bile depletion markedly downregulated the protein levels of P-LKB1 during the first 3 days after ligation, which might be responsible for the decrease of hepatocyte polarization (fig. 4). Meanwhile, the MEK-LKB1-AMPK signaling pathway activation in the tPVL group mimicked the sequential activation observed in the BPL group (fig. 4).

3 DISCUSSION

The present study demonstrated that compared to PVL, BPL could accelerate reconstruction of the CN and induce proper pre-activation of the polarity-establishing signaling pathway. After the staged hepatectomy, the remnant liver manifested prompt

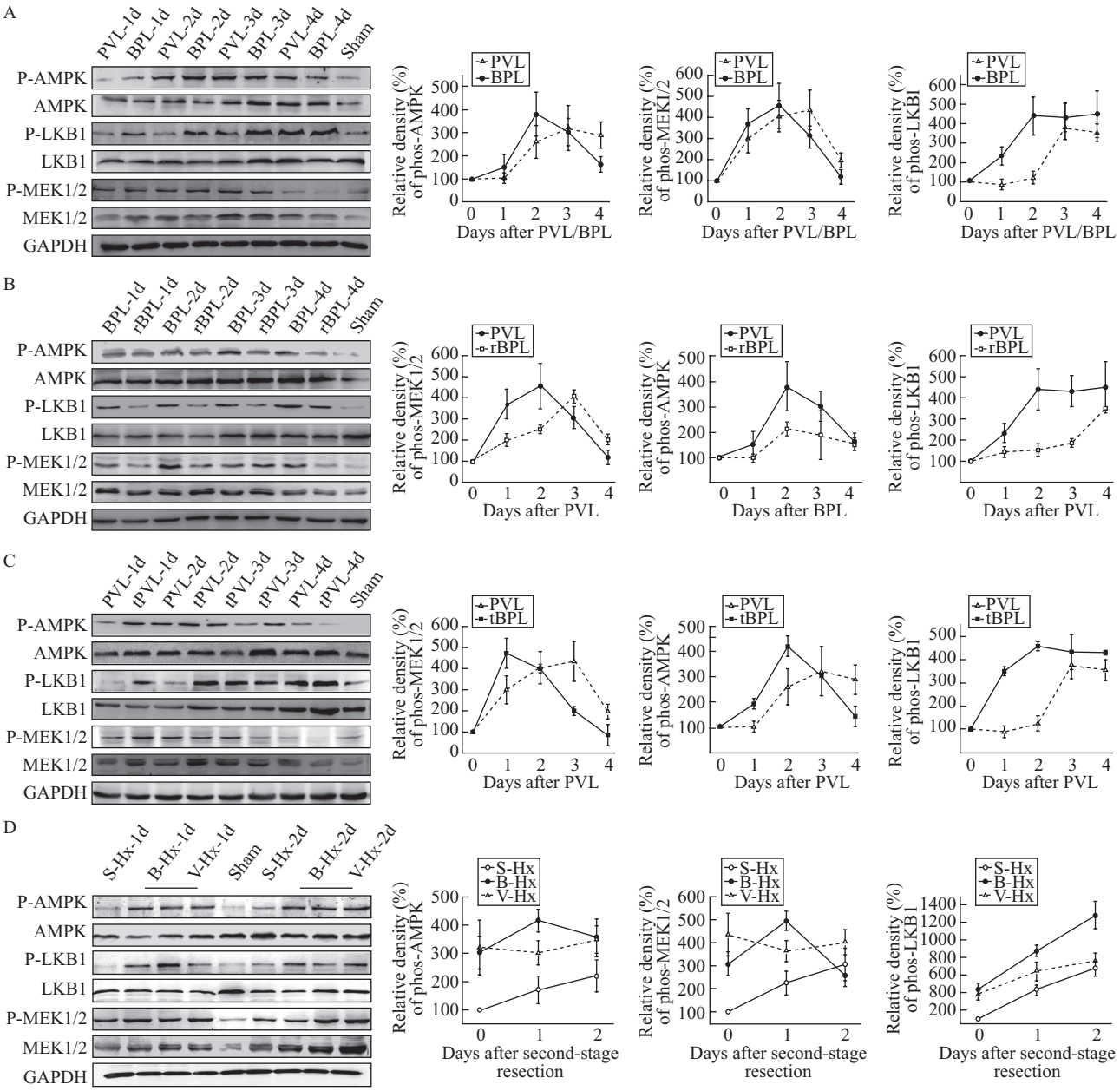

Fig. 4 Protein levels of total and phos-AMPK (Thr-172), total and phos-LKB1 (Ser-431), and total and phos-MEK1/2 (Ser217/221) after BPL or PVL (A), after BPL or rBPL (B), after PVL or tPVL (C) and in the S-Hx, V-Hx, and B-Hx groups (D). The data are presented as mean ± SD.

hepatocyte polarization and limited CN disturbance, which gave rise to favorable survival. Furthermore, by manipulating the BA retention in the BPL and PVL models, respectively, we provided direct evidence that enhanced BA retention underlies the effects induced. Our study revealed that BPL as a safe and appealing procedure could significantly improve the prognosis of rats receiving an extended hepatectomy and proved that enhancing BA retention could promote hepatocyte polarization and CN reconstruction *in vivo*.

Previously, we demonstrated that compared with PVL, BPL significantly promoted the regeneration of the intact liver by enhancing BA retention, and the increased volume of the FRL guaranteed the survival benefit after a staged hepatectomy[7]. However, in the current study, a staged hepatectomy after BPL still resulted in a favorable survival without a superior FRL, ruling out liver weight as the main reason for the distinct survival rates. An extended hepatectomy is associated with a poor regenerative response and unbalanced regeneration[28, 29]. PVL is believed to improve the tolerance of the hypertrophied FRL to an extended hepatectomy, and earlier initiation of proliferation was reasoned as the underlying mechanism[30]. In the present study, stable and robust hepatocyte proliferation was consistently observed in both the V-Hx and B-Hx groups, and the differences were too minor to be able to explain the large differences in survival, indicating that some other mechanisms were involved. During the early phase after partial hepatectomy, the remaining hepatocytes are exposed to an increased BA load, but thereafter the BA levels rapidly decrease within 48 h along with reconstruction of the CN[22, 31]. Prolonged loss of canaliculi and CN disturbance have been proposed as causes of liver failure, and ameliorating these conditions improves survival in rats[16, 17]. In the current study, an activated LKB1-AMPK-MEK signaling pathway coexisted with a reconstructed CN in the B-Hx group at the time of resection. A pre-activated signaling pathway led to the most prompt polarization and the least disturbance of the CN after the staged resection. In addition, energy metabolism usually improves progressively, in parallel with polarization[32, 33], implying that the superior energy metabolism might also have contributed to the improved tolerance to resection in the B-Hx group.

Bile canaliculi are formed by the apical domains of polarized adjacent hepatocytes[10]. The presence of a well-branched CN depends on polarized hepatocytes arranged in a defined pattern. For regeneration of almost any kind, dedifferentiation and tissue remodeling are inevitable[34, 35]. Liver regeneration is no exception[36]. The neogenetic hepatocytes are poorly polarized, and the thickening of hepatic plates disrupts the pre-existing CN. It is impossible to avoid hepatocyte depolarization, and the disturbance of the CN likely aggravates paralleling the speed of regeneration[17]. Hence, as observed in our study, the faster regeneration induced by BPL might presumably intensify the disturbance compared to after PVL. Yet as BAs were dispensable during liver regeneration for the re-establishment of hepatocyte polarity and reconstruction of the CN[1, 2, 10, 18–20], the attenuated BA retention in PVL may have led to the prolonged loss of the CN. While in the BPL group, the ligation of the bile duct intensified the BA retention in the intact liver, which potently promoted the polarization and maturation of the newly generated hepatocytes. These hypotheses were further supported by the prolonged CN disturbance in the rBPL group, in which we diminished hepatic BA in the BPL model, and by the accelerated CN reconstruction in tPVL, in which we increased hepatic BA in the PVL model. These findings were further verified by the timing and degree of activation of the LKB1-AMPK-MEK pathway. Our study proved that restoration of the cellular polarity and reconstruction of the CN during liver regeneration could be accelerated *in vivo* by BAs.

In recent years, BA signaling and BA pool composition are increasingly considered crucial for liver pathophysiology[37–40]. It must be noted that enhancing the BA overload by supplementation could be deleterious, especially after an extended hepatectomy. A recent study found that the postextended hepatectomy survival upon ursodeoxycholic acid treatment was strongly reduced in mice[41], consistent with studies reporting that ursodeoxycholic acid may aggravate obstructive cholestasis in several experimental and clinical settings[42]. This study also uncovered that TGR5-dependent control of BA overload and BA composition is critical for the posthepatectomy outcome, and shifting the BA pool composition toward less toxic BAs was correlated with increased survival rates, whereas a more hydrophobic BA composition was associated with more severe posthepatectomy outcomes. In the current study, the enhancement of BA overload was achieved by BPL without direct intervention on the BA pool composition. We assumed that BPL preconditioned the FRL, which may have triggered strong TGR5 activation, thus contributing to a beneficial BA pool composition. This should be further investigated in future studies.

Of note, the components of the BA pool differ significantly between rodents and humans[43]. For example, ursodeoxycholic acid treatment in mice, in complete opposition to humans, increases the BA pool hydrophobicity, pointing out species differences in BA metabolism[44]. The BA pool of humans is more hydrophobic, which might cause more severe liver damage[45]. The BA pool composition before a major hepatectomy is critical for patients after surgery[41]. Whether this model could be reproduced in humans is unknown. Intriguingly, we found that combining

the oral administration of taurocholate with PVL led to remarkably accelerated hepatocyte polarization. Compared with BPL, oral ingestion seems more attractive, since the need for surgical interventions would be minimized. Further studies should focus on identifying a proper dosage and the optimal kind of BA to induce such an effect[22].

Although our previous study and a case report have consistently demonstrated that BPL could enhance the hypertrophy of the intact liver with a satisfactory safety profile[7, 9], the more complicated procedure of BPL and the relatively limited hypertrophic effect may still make BPL a less appealing option. Our study demonstrated that in addition to increasing the FRL, BPL shortened the duration of the spatial disturbance of the CN by 24 h and could significantly improve the tolerance of the hypertrophied liver to staged resection. Therefore, we propose that BPL may be a safe and efficient future option for patients with an insufficient FRL.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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