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Simultaneous hepatic and portal vein ligation induces rapid liver hypertrophy: A study in pigs

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ABSTRACT

Background: Liver hypertrophy induced by partial portal vein occlusion (PVL) is accelerated by adding simultaneous parenchymal transection ("ALPPS procedure"). This preclinical experimental study in pigs tests the hypothesis that simultaneous ligation of portal and hepatic veins of the liver also accelerates regeneration by abrogation of porto-portal collaterals without need for operative transection.

Methods: A pig model of portal vein occlusion was compared with the novel model of simultaneous portal and hepatic vein occlusion, where major hepatic veins draining the portal vein–deprived lobe were identified with intraoperative ultrasonography and ligated using pledgeted transparenchymal sutures. Kinetic growth was compared, and the portal vein system was then studied after 7 days using epoxy casts of the portal circulation. Portal vein flow and portal pressure were measured, and Ki-67 staining was used to evaluate the proliferative response.

Results: Pigs were randomly assigned to portal vein occlusion ($n = 8$) or simultaneous portal and hepatic vein occlusion ($n = 6$). Simultaneous portal and hepatic vein occlusion was well tolerated and led to mild cytolysis, with no necrosis in the outflow vein–deprived liver sectors. The portal vein–supplied sector increased by 90 ± 22% (mean ± standard deviation) after simultaneous portal and hepatic vein occlusion compared with 29 ± 18% after PVL ($P < .001$). Collaterals to the deportalized liver developed after 7 days in both procedures but were markedly reduced in simultaneous portal and hepatic vein occlusion. Ki-67 staining at 7 days was comparable.

Conclusion: This study in pigs found that simultaneous portal and hepatic vein occlusion led to rapid hypertrophy without necrosis of the deportalized liver. The findings suggest that the use of simultaneous portal and hepatic vein occlusion accelerates liver hypertrophy for extended liver resections and should be evaluated further.

Introduction

Portal vein occlusion by ligation (PVL) or embolization is a well-established method to improve the safety of extended liver resections by inducing liver hypertrophy before resection.[1–3] The novel procedure associating liver partition and portal vein ligation (ALPPS) indicated that kinetic growth of the future liver remnant

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can be increased by adding a parenchymal transection between the portal vein–supplied and portal vein–deprived part of the liver.[4] A recent pig study from our laboratory suggested that the parenchymal transection in ALPPS leads to an abrogation of extensive porto-portal neocollaterals between the portal vein–supplied and the portal vein–deprived side of the liver.[5] Porto-portal collaterals are common in PVL and also occur in portal vein embolization; these collaterals are known to blunt hypertrophy, and their complete abrogation may well be the cause for the astounding effectiveness of parenchymal transection in ALPPS. Based on the concept that abrogation of collaterals is a key requirement for rapid hypertrophy, we postulated that simultaneous occlusion of hepatic and portal veins may abrogate the formation of porto-portal collaterals to the deportalized side of the liver and thereby accelerate hypertrophy compared with PVL. This study tested whether simultaneous ligation of portal veins and hepatic veins (PLV+HVL) to the deportalized side of the liver would accelerate the increase in liver volume of the contralateral nondeportalized liver in a pig model.

Methods

Ethical statement

Approval for the experiments was obtained from the Internal Animal Care and Use Committee of Rush University Medical Center (No. 15-025). The experiments were performed in compliance with the Guide for the Care and Use of Laboratory Animals by the National Research Council, 2011 edition (https://www.nap.edu/download/12910), and the ARRIVE guidelines.[6]

Study design and allocation

Pigs were allocated randomly to undergo either PVL or PVL+HVL. The primary endpoint of the study was the increase in liver volume of the part of the liver still supplied by the portal vein and the development of collaterals after 1 week. In addition, data from a previously published study[5] were used to compare the increase in liver volume after PVL and PVL+HVL with the increase in liver volume in a large animal (pig) model of ALPPS.

Experimental animals and housing

Female Yorkshire Landrace pigs were obtained from Oak Hill Genetics (Oak Hill, IL). The pigs were kept in pairs in standard, pathogen-free conditions in an alternating 12-hour light/dark cycle at least for 7 days for accommodation. Food and water were provided ad libitum, and the ambient temperature was 22 ± 1°C. The animals were weighed before the procedures and after 7 days when they were re-explored; the livers were procured at this time. The operative procedures were always performed in the mornings between 9:00 am and 12:00 am. After operation, the animals were housed individually to avoid tampering with their tunneled venous catheters.

Sample size

Based on previously published experiments, we included 14 animals in the study in order to being able to study more than 5 pigs per group in the random allocation. In a previously reported study, the difference in volume growth (mean ± standard deviation) between ALPPS ($n = 4$; 63 ± 14%) and PVL ($n = 4$; 16 ± 9%) was 48% with what appeared to be a normal distribution.[7] Assuming a power of 0.8 and a standard deviation of 0.1 and a difference to detect of 0.48, at least 5 animals per group were necessary to determine if the novel procedure of PVL+ HVL induces as much hypertrophy as ALPPS. Sample size calculation was performed using JMP by SAS (SAS Institute, Inc, Cary, NC).

Experimental procedures: Anesthesia

A 25-μg fentanyl patch was placed on each pig the evening before operation. On the day of operation, the animals were premedicated with a single IM intramuscular injection of telazol and xylazine dosed from a weight-based medication table and given atropine (0.09 mL/kg, intramuscular injection). After inserting peripheral IV catheters into each ear, the pigs were intubated using 5-mm endotrachial tubes. Animals were ventilated with a Jorvet SAV 2500 veterinary ventilator (Smiths Medical PM, Inc., Norwell, MA) at 15 breaths/min using a Jorvet Isotec 4 nebulizer with Isoflo (Abbot Laboratories, North Chicago, IL), 1.0–3.0 vol% vaporized in 600 mL oxygen/min. An open cut-down was performed to the external jugular vein to insert a tunneled, 2-lumen central venous catheter (Leonard central venous catheter, C. R. Bard, Inc, Murray Hill, NJ) as well as an arterial line (Arrow, Teleflex Medical, Morrisville, NC) into the right carotid artery. Arterial pressure was monitored using a GE Transport Pro monitor (GE Medical Systems, Milwaukee, WI). Volume resuscitation was maintained using a Flo-Guard 6300 dual-channel volume infusion pump (Baxter International, Inc, Deerfield, IL), keeping a strict central venous pressure of 5 mm Hg to standardize hemodynamic measurements during the procedure. After the procedures, the arterial line was removed by ligation of the carotid artery and both limbs of the tunneled central venous catheter were locked with 1,000 U/mL heparin sulfate to prevent blood clots and allow blood draws in the postoperative period.

Experimental procedures: Operation

The pig liver consists of 5 sectors supplied by the portal vein (right lateral [RL], right medial [RM], left medial [LM], left lateral [LL], and caudate lobe [CL]), which are only partially congruous with the externally visible lobulation (Fig. 1, *A*).

PVL was performed using the RL sector as described previously (Fig. 1, *B*).[5] Methylene blue (10 mL) was injected into the portal vein to identify the demarcation line to the RL sector before marking its borders with electrocautery.

HVL+PVL was performed in addition to portal vein ligation (Fig. 1, *C*). The hepatic veins draining the RM, LM, and LL sectors were identified using intraoperative ultrasonography (Fig. 1, *D*). Double-armed CTX no. 1 polypropylene sutures were used to suture around each vein under ultrasonographic guidance with quadruple-folded umbilical tape threaded on the suture to serve as a pledget on the liver surface (Fig. 1, *E*), and the sutures were tied down slowly until cessation of flow could be confirmed by ultrasonography (Fig. 1, *F*). The abdomen was then closed using no. 1 polydioxanone sutures, skin was closed with 3-0 polyglactin, the intravascular lines were removed except for the double-lumen Leonard catheter, and the animals were recovered.

A laparotomy was performed 7 days later, again under general anesthesia. Methylene blue (10 mL) was again injected into the portal vein to test for demarcation on the liver surface. After ultrasonographic and photographic documentation, 2 L of 0.9% NaCl were used to perfuse the liver using aortic cannulation for the liver procurement.

Primary outcome: Volume increase

The right lateral sector was weighed using Archimedes' principle of immersion weight shift using the demarcation line of the posterior sector as the immersion line as described before.[7] The volume of the future liver remnant measured after 7 days

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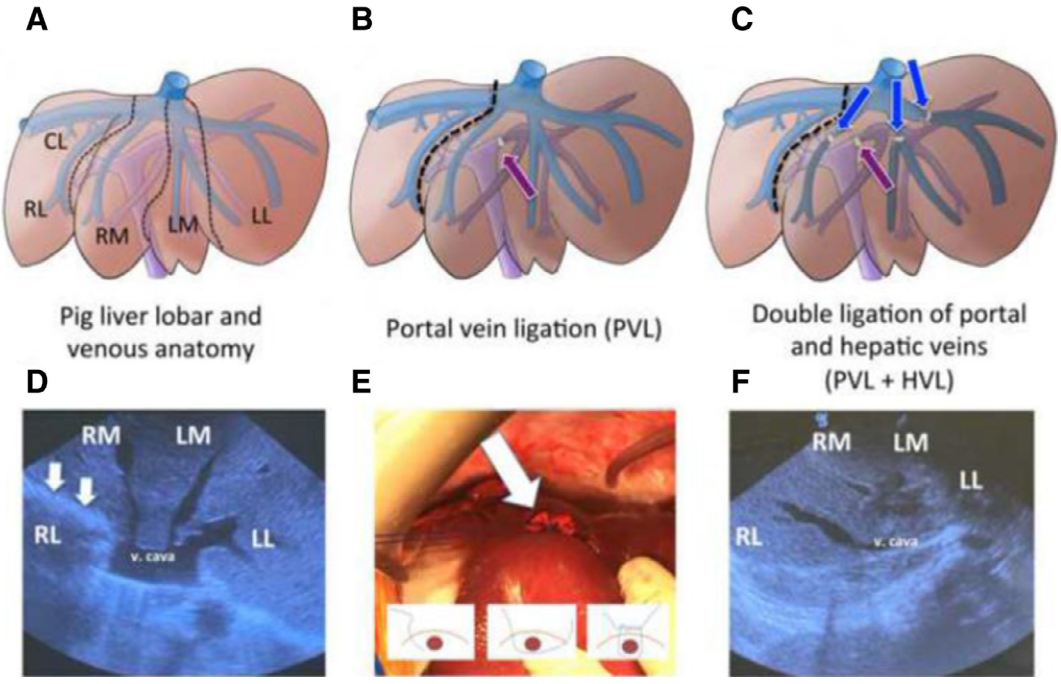

Fig. 1. Experimental procedures in pigs. (A) Portal vein anatomy of the pig liver. The right lateral sector (RL), caudate lobe sector (CL), right median sector (RM), left median sector (LM), and left lateral sector (LL) are shown. The portal venous supplies to each sector are not congruent with the borders of the external lobulation, a common theme in liver anatomy. The watersheds between sectors cross the lobes and tissue bridges between lobes in a line with a sigmoid shape (dotted line). (B) Portal vein ligation (PVL) procedure leads to an acute cessation of portal vein flow to the RM, LM, and LL and portal hyperflow to the RL and CL. The ligation of the portal vein distal to the takeoff of the RL lobe branch (*arrow*) is achieved using portal pedicle dissection and a silk tie. (C) Portal vein ligation plus hepatic vein ligation procedure (PVL+HVL). In PVL+HVL, in addition to PVL, the 3 hepatic veins draining the RM, LM, and LL are additionally ligated using long and large XLS needles with no. 1 polypropylene sutures and pledgets. The RM, LM, and LL veins are ligated. (D) Intraoperative ultrasonography is used to identify the 3 veins draining the RM, LM, and LL. A sponge is placed between the right lobe and the right median lobe to delineate a line of echogenicity between the lobes (*white arrows*) to safely spare the RL vein. (E) Intraoperative photograph of PVL+HVL. The photo shows the representative placement of a venous occlusion stitch with the white umbilical tape serving as a pledget. The 3 inserts show the steps of the procedure; the depth of the needle tract is determined by ultrasonography. (F) Photograph of intraoperative ultrasonography. At the end of the PVL+ HVL procedure, intraoperative ultrasonography confirms the occlusion of the RM, LM, and LL veins and persistence of a patent RL vein that drains into the vena cava.

was compared with an assumed baseline volume calculated from weight of each individual pig and to the median liver–to–body weight index of the RL in normal pigs of 0.46 as described before.[7] Growth of the posterior liver sector after 7 days was measured directly and expressed in absolute volume increase in milliliters and also in relative increase in percentages.

Experimental outcomes: Portal vein casts

Epoxy resin (Batson Anatomical Corrosion Kit, Polysciences, Inc, Warrington, PA) was used; a red color was used to cast the portal vein system and a blue color to cast the hepatic vein system as described before.[5] Of the 8 pig livers after PVL, 4 casts were performed; 4 others were used for histologic analyses of the collateral system (not reported here). Of the 6 pig livers after PVL+HVL, 5 casts of the portal vein system and 1 arterial cast were made. A Vernier digital microcaliper (Mitutoyo, Aurora, IL) was used to measure the diameter of collaterals.

Experimental outcomes: Hemodynamic parameters

For hemodynamic measurements, central venous pressure was kept at 5 mm Hg by graduated fluid resuscitation. Portal flow was assessed using flow probes (Transonic Europe BV, Elsloo, the Netherlands), and portal vein pressure was assessed using pressure transducers as described before.[7] Portal flow was quantified as portal flow per cubic centimeter liver tissue, and portal pressure was expressed as porto-caval pressure gradient across the liver.

Experimental outcomes: Laboratory values

Blood chemistry, hematology, liver panel, and coagulation parameters were determined daily as described before.[7]

Experimental outcomes: Histology

Liver tissue was obtained by wedge biopsy at baseline after entering the abdomen and after 7 days immediately after entering the abdomen. Tissue was fixed, embedded, and stained with hematoxylin-eosin Ki-67 for immunohistochemical analysis as described before.[7] Differently from our previous study,[7] the number of Ki-67–positive cells was determined by counting the number of Ki-67–positive hepatocytes per 1,000 hepatocytes by excluding of Kupffer, stellate, and endothelial cells.[5] ImageJ software (National Institutes of Health, Bethesda, MD) was used for calibration of histologic testing.

Statistical methods

Results are expressed for parametric values as mean and standard deviation and for nonparametric data as median and range. The Kolmogorov-Smirnov test was used for normally distributed data. For comparison of groups, analysis of variance was used for parametric data, and Kruskal-Wallis or Friedmann tests were used for nonpaired and paired nonparametric data. XY statistical testing was performed using linear regression. Details on descriptive and comparative statistics used are given in each figure legend. Prism 6.0 (GraphPad Software, Inc, San Diego, CA) was used for data analysis and presentation.

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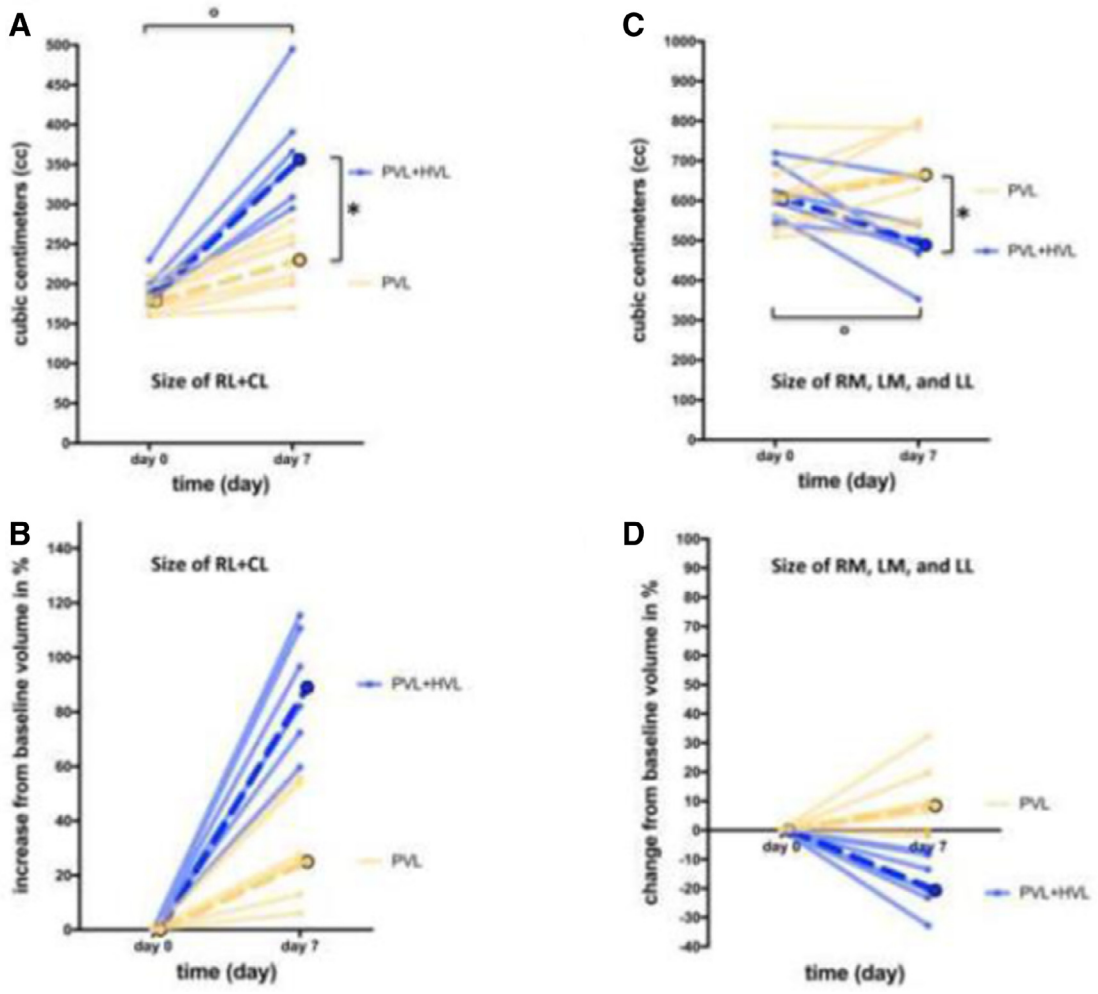

Fig. 2. Volume changes of the pig liver over 7 days after portal vein ligation (PVL) and PVL plus hepatic vein ligation (PVL+HVL). (A) Absolute volume changes of sectors supplied by the portal vein (right lateral and caudate lobe [RL+CL]) in cubic centimeters over 7 days. The volume of the sectors supplied by the portal vein (RL+CL) increases significantly after PVL+HVL ($^{\circ}P < .001$) but not significantly after PVL ($P = .14$). The difference in size between the lobe supplied by the portal vein 7 days after PVL and 7 days after PVL+HVL is different ($^{*}P < .001$). Data are normally distributed by Kolmogorov-Smirnov test, and 1-way analysis of variance was used for comparison of 4 groups. (B) Relative change in volume in the sector supplied by the portal vein in percentages. The graph shows the relative increase in volume of the sector supplied by the portal vein after both procedures compared with baseline. (C) Absolute changes in volume of the sectors deprived of portal venous inflow (right median, left median, and left lateral sector [RM+LM+LL]). The liver deprived of portal venous inflow (RM+LM+LL) decreases in size after PVL+HVL ($P < .001$), whereas it does not change after PLV alone ($P = .10$). There is a significant difference between the size of the deportalized sector after PVL and PVL+HVL ($P < .001$). Data are normally distributed by Kolmogorov-Smirnov test, and 1-way analysis of variance was used for comparison of 4 groups. (D) Relative change in the volume of the sectors deprived of portal venous inflow in percentages. The graph shows the decrease in the volume of the liver sectors deprived of portal venous inflow after PVL+ HVL compared with PVL.

Results

Outcome: Volume changes

The absolute increases in volume 7 days after the respective procedures for each animal are shown in Fig. 2, *A*, with the mean increase marked in bold. Figure 2, *B*, shows the same volume increase in percentages. Compared with baseline volume, the portal vein–supplied sector increased by 90 ± 22% after PVL+HVL ($P < .001$), whereas the volume increase 7 days after PVL was 29 ± 18%, which was not statistically significant. The difference between PVL+HVL and PVL at 7 days was 3.1-fold. The liver deprived of portal flow remained unchanged after PVL, but decreased significantly in volume after PVL+HVL (Fig. 2, *C*). Figure 2, *D*, shows the volume change of the deportalized sectors for each pig in percentages with the mean in bold. The volume loss after PVL+HVL was consistent at a mean of 18 ± 8%, whereas after PVL, some of the livers deprived of portal flow increased and others decreased in volume relative to baseline.

Outcome: Liver surface staining with methylene blue

At the time of operation (day 0), the injection of methylene blue showed the characteristic demarcation of the sectors going across the 2 external lobulations (Fig. 3, *A*). At the time of reoperation on postoperative day 7, a blue demarcation line was largely absent in PVL and there was staining across the liver with no clear demarcation line (Fig. 3, *B*). In contrast, RL+CL stained intensely 7 days after PVL+HVL with the demarcation line shifted somewhat medially (Fig. 3, *C*).

Outcome: Epoxy casting of the portal vein system

A representative example of 4 epoxy casts 7 days after PVL is shown in Fig. 4, *A*. The portal vein system of the portal vein–deprived liver, although completely disconnected from the main portal vein, filled from the portal vein system supplying the RL+CL sector through dense collaterals, each with a diameter of between 0.5–4 mm across the watershed to the left portal vein system as

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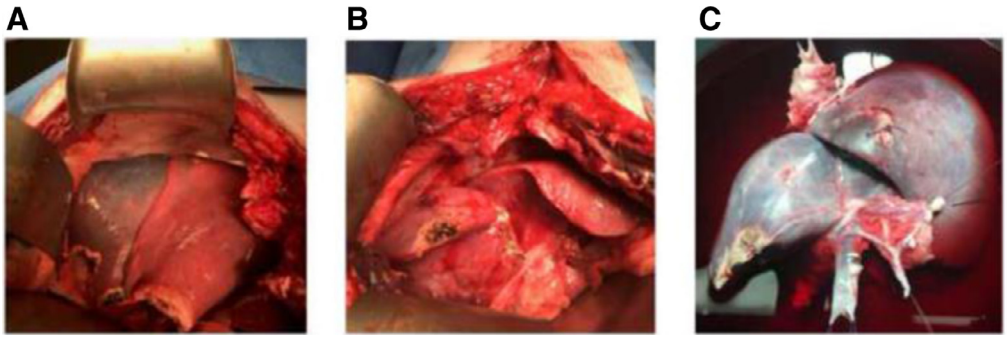

Fig. 3. Methylene-blue staining after injection of 10 mL methylene blue into the main portal vein (A) at baseline, (B) 7 days after PVL and (C) 7 days after PVL plus hepatic vein ligation (PVL+HVL). (A) The sigmoid shape of the portal supply area of right lateral and caudate lobe sector (RL+CL) is delineated by the blue staining of the watershed. (B) There is no intraoperative line of demarcation 7 days after PVL because the methylene blue distributes across the entire liver, likely through the collaterals that have developed. (C) There is a line of demarcation after PVL+HVL, somewhat shifted to the right median sector when compared with the baseline in (A).

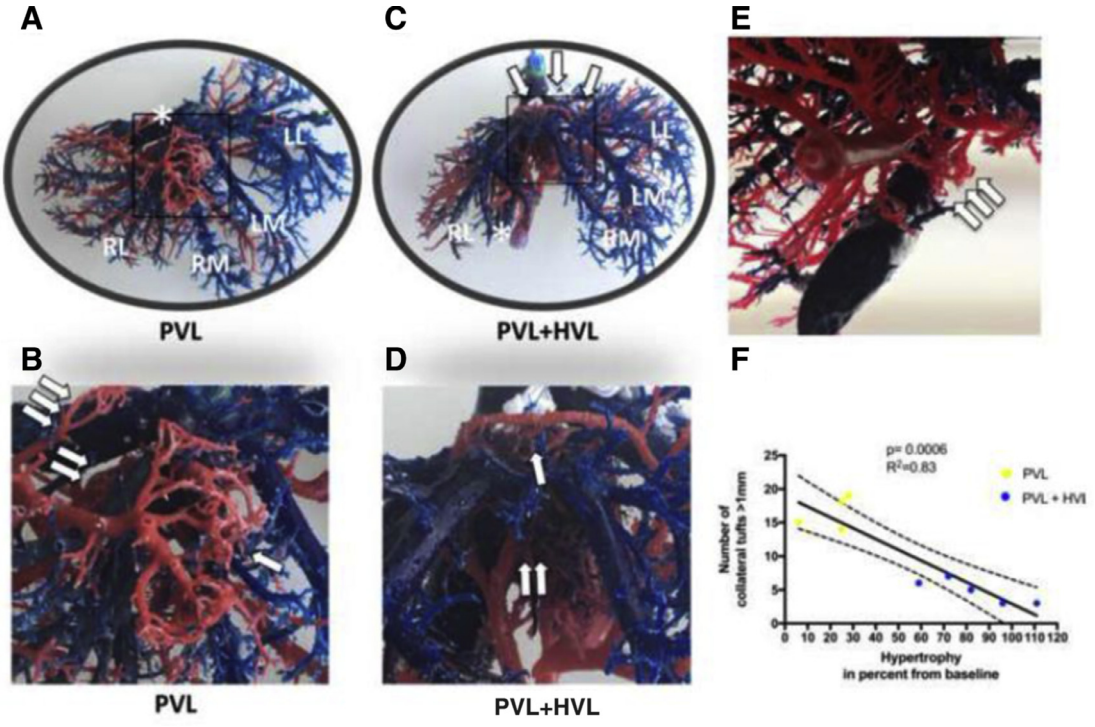

Fig. 4. Evaluation of portal vein collaterals using epoxy casts of the portal vein system (red resin) and the hepatic veins (blue resin) of entire pig livers 7 days after portal vein ligation (PVL) and PVL plus hepatic vein ligation (PVL+HVL). (A) Epoxy cast of pig liver 7 days after PVL shows the right lateral sector (RL), the right median sector (RM), the left median (LM), and the left lateral sector (LL) of the pig liver, with the blue resin showing the hepatic veins and the red resin the portal vein system. There are extensive neocollaterals (red resin) across the normally avascular watershed between the RL and the RM giving rise to secondary filling of the portal venous tree of the RM, LM, and LL. The vena cava (blue) is deflected posteriorly and marked by asterisk (*). The square outlines the enlarged area in (B). (B) The enlargement of the frame in (A) shows the 3 main groups of collaterals identified in the PVL cast (1 arrow, red) anterior to the RL vein (2 arrows, red), posterior to the RL vein, and (3 arrows, red) through the caudate lobe. (C) Epoxy cast of pig liver 7 days after PVL+HVL. The extensive neocollateralization between the RL and RM identified after PVL is here notably absent except for very few collaterals. The vena cava (blue) is deflected inferiorly and marked by asterisks (*). The 3 pledgets made from umbilical tape and used to reinforce the venous ligation sutures on the liver surface are visible even after the digestion process of the liver tissue. The ligated hepatic veins, however, have recanalized. (D) The enlargement of the frame in (C) shows a >1 mm diameter neocollateral (red) giving off multiple small branches (1 arrow). In addition, a group of collaterals can be seen posterior to the RL hepatic vein (red, 2 arrows). (E) The caudate lobe collaterals 7 days after PVL+HVL are best visualized from an inferior perspective (red, 3 arrows). (F) Correlation graph of collaterals measured by a Vernier digital microcaliper to be >1 mm and percentage volume increase in 4 epoxy casts from pig livers 7 days after PVL (yellow) and 6 epoxy casts from pig livers 7 days after PVL+HVL (blue). There is a significant negative correlation (glomerular tufts = $-0.1605 \times$ % hypertrophy + 18.99) with an R^2 of 0.83.

reported before.[7] Three systems of collaterals were identified, 1 anterior to the right hepatic vein (Fig. 4, *B*, 1 arrow), 1 posterior to the right hepatic vein (Fig. 4, *B*, 2 arrows), and 1 through the caudate lobe (Fig. 4, *B*, 3 arrows). In the epoxy cast model 7 days after PVL+HVL, collaterals had also developed across the watershed but were smaller and less pronounced (Fig. 4, *C*). All 3 collateral systems that were identified in PVL were present but with fewer and smaller-sized collaterals. Collaterals anterior (Fig. 4, *D*, 1 arrow) and posterior (Fig. 4, *D*, 2 arrows) to the right hepatic vein were found, as well as through the caudate lobe that wraps around the vena cava and connects the RL to the RM lobe in pigs (Fig. 4, *E*, 3 arrows). Quantification of collateral development after 7 days was performed by counting the number of collaterals larger than 1 mm in diameter and plotting them on a correlation graph (Fig. 4, *E*). There was a good correlation ($R = .83$, $P < .001$) between the number of collaterals >1 mm and the volume increase in volume of the portal vein–supplied sector within 7 days.

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Outcome: Hemodynamic changes in PVL and PVL+HVL

Volume measurements of portal vein flow before and 1 hour after performing the ligations of PVL or PVL+HVL had no significant difference in main portal vein flow after 1 hour or at 7 days later when the livers were procured. This indicated that in pigs the same mesenteric blood volume is pushed through the portal supplied part of the liver after the ligation procedures as is flowing through the entire liver before, as has been reported in previous experiments in our laboratory in rats[8] and pigs.[7] The index of volume of portal blood flow (in ml) per volume liver tissue (in cubic centimeters) per minute increased from 1.06 (interquartile range [IQR] 0.83–1.25) mL/cm^3/min and 1.29 (IQR 1.14–1.36) mL/cm^3/min to 3.69 (IQR 2.93–5.39) mL/cm^3/min and 5.68 (IQR 4.61–5.96) mL/cm^3/min in PVL and PVL+HVL, respectively, after the ligation procedures on day 0. There was no difference in the flow changes between PVL and PVL+HVL. The index remained increased after 7 days, if the hypertrophying portal vein–supplied liver remnant is in the denominator as shown in Fig. 5, *A*. If the portal blood, however, perfuses a larger part of the liver, as suggested by the collaterals found in the epoxy casts, the denominator increases in a way that is difficult to determine, making the true index difficult to assess. The porto-caval gradient in mm Hg for PVL and PVL+HVL at baseline was 1 (IQR 1–2.75) mm Hg and 2.5 (IQR 1.75–3) mm Hg and increased to 6.5 (IQR 5.25–7.75) mm Hg and 5.5 mm Hg (IQR 4.75–9.25), respectively ($P = .008$ and $P = .007$, respectively) after the flow restriction to about 21% of the previous liver volume (Fig. 5, *B*). The acute increase of the pressure gradient returned to normal after 7 days in both procedure types. There was no difference in the acute portal hypertension induced by PVL and PVL+HVL.

Outcome: Serum hepatocyte injury markers and liver function tests

The animals were well, no animal died prematurely, and no animal had ascites on re-exploration. The deportalized and the hypertrophying liver looked viable on re-exploration on day 7 in both groups. Figure 5, *C*, shows that aspartate aminotransferase levels were increased ($P = .004$) after both PVL and double-ligation PVL+HVL and remained so until day 3, when they returned to normal. There was, however, no difference between PVL and PVL+HVL. Alanine aminotransferase remained unchanged and did not differ between procedures (Fig. 5, *D*). The metrics of liver transfer function for total bilirubin was very low in pigs at baseline and remained unchanged (Fig. 5, *E*). The metrics of liver synthetic function as evaluated by the international normalized ratio and prothrombin time also remained unchanged and did not differ between procedures (Fig. 5, *F*).

Outcome: Histologic changes

Figure 6, *A,* shows the normal portal tract anatomy in pigs with normal-sized portal veins. Figure 6, *B* and *C,* show dilated portal veins after 7 days in the portal vein–supplied hypertrophying lobes after PLV and PVL+HVL, respectively. Quantitative evaluation of Ki-67 staining at baseline (Fig. 6, *D*), 7 days after PVL (Fig. 6, *E*), and 7 days after PVL+HVL (Fig. 6, *F*) indicated no significant changes (Fig. 6, *G*). The liver lobe deprived of portal inflow and venous outflow did not have any signs of tissue degeneration in × 100 magnification (Fig. 6, *H*) inflammatory infiltrates, or cellular degeneration in × 600 magnification (Fig. 6, *I*).

Discussion

In a preclinical large animal model, this study found that the simultaneous double ligation of portal and hepatic veins induced 3 times the amount of liver hypertrophy compared with portal vein ligation alone within 7 days. This simultaneous double ligation maneuver of PVL+HVL is surprisingly well tolerated, does not lead to excessive increases in transaminase suggestive of hepatocyte injury compared with PVL alone, and does not produce any laboratory evidence of liver dysfunction as evidenced by serum total bilirubin or international normalized ratio. Despite the outflow obstruction of all major veins of the deportalized liver, there was no evidence of hepatocyte necrosis on histologic examination, likely because of the remaining arterial flow. The amount of hypertrophy induced by this double ligation technique is at least as extensive as the rapid hypertrophy in a pig model of ALPPS, where a 64% increase in liver volume was seen at 7 days.[7] Both methods led to a comparable increase in volume of the portal vein–supplied sector compared with PVL alone.

The mechanism of the identified effect of double ligation on hypertrophy remains unclear. We speculate that the inflow of hepatotrophic growth factors through collaterals can be abrogated either by transection of the parenchyma or by additional ligation of the hepatic veins in addition to the portal veins. We propose that the mechanism is abrogation of collateral flow bringing the substances that have in the past been described as the "hepatotrophic growth factors," such as insulin, insulin-like growth factor, epithelial growth factors, and so on, to the growing liver.[9] In the ALPPS model in pigs, the area of portal vein perfusion in ALPPS after 7 days, as indicated by the methylene blue coloring, was confined to the right lobe along the line of parenchymal transection (not shown).[7] Similarly, 7 days after PVL+HVL, the area of portal vein perfusion remained restricted to the right lobe (Fig. 3, *C*), whereas it dissipated across the entire liver in the PVL group (Fig. 3, *B*).

The hepatic hypertrophy of the double ligation approach in a preclinical pig model is surprising because sequential hepatic vein occlusion in the past has been found to be only moderately effective, likely because hepatic vein occlusion has been used previously as a salvage maneuver in a sequential fashion after portal vein occlusion.[10,11] The profound effect on hypertrophy of the remnant hepatic volume may be the result of the simultaneous occlusion of both systems.[12,13] It may be that an initial growth stimulus matters, but without portal escape, collaterals matter and sequential embolization does not provide that stimulus. Interestingly, also, simultaneous portal vein and hepatic vein occlusion was attempted in a preclinical rabbit model in the past but was found to yield a hypertrophy response that was not considered superior to portal vein embolization alone.[14] The findings may be explained by the anatomic specificities of the rabbit—that is, the almost complete absence of potential collateral flow within the organ the parenchyma in the highly lobulated rabbit liver.[15] The anatomy of the rabbit liver does not lend itself to studying the effects of collateral abrogation because the liver is much more extensively lobulated than the pig liver. After embolization or ligation of the left or distal liver, the potential for development of collaterals is minimal because there is only a diminutive parenchymal tissue mass in continuity with the deportalized and the growing liver. The disappointing findings in patients and in this specific animal model may have discouraged others from pursuing a simultaneous double occlusion of both portal and hepatic veins.

Recently, however, the simultaneous embolization (rather than ligation) of portal and hepatic veins has been described in humans in a pilot series under the name of "liver venous deprivation" technique, with results that suggest that simultaneous double embolization in humans also induces rapid hypertrophy.[12,13]

Although ALPPS currently garners a lot of attention because it allows induction of rapid hypertrophy before resection with the promise to expand resectability of liver tumors with a large tumor load,[4,16,17] ALPPS as a routine procedure remains controversial.[18–20] ALPPS has a high perioperative morbidity and mortality rate.[21,22]

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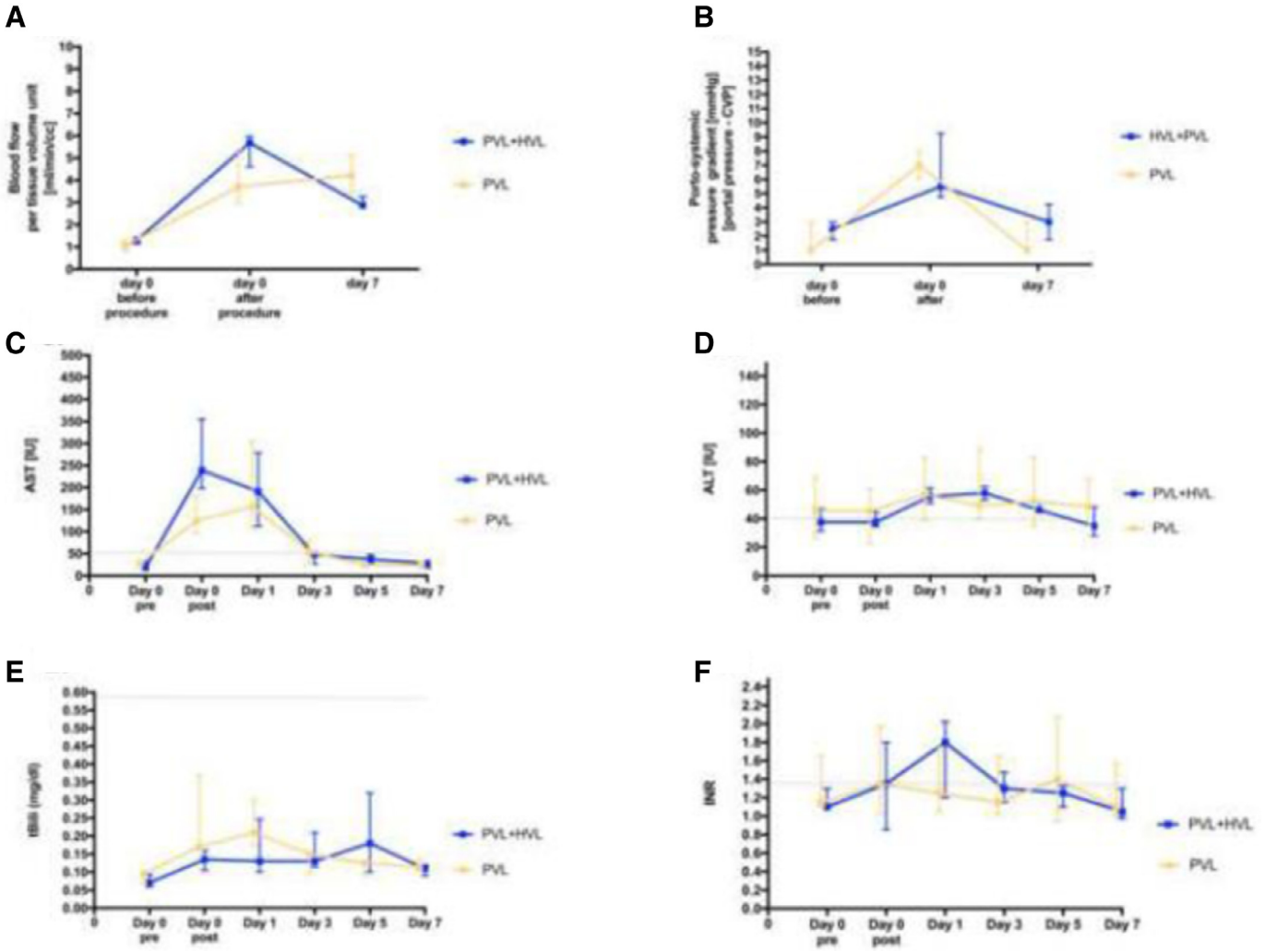

Fig. 5. Hemodynamic and laboratory changes after portal vein ligation (PVL) and PVL plus hepatic vein ligation (PVL+HVL). (A) Volume of flow in the main portal vein corrected for the volume of liver tissue perfused. Intraoperative direct measurements of flow in the main portal vein flow corrected for the estimated tissue volume perfused based on estimated liver weight indicate an increase in portal vein flow per tissue immediately after the procedure for both PVL ($P = 0.017$) and PVL+HVL ($P = .002$). There are no differences between PVL and PVL+HVL for all measurements. The perfused volume after 7 days was the weighed hypertrophied liver. Given the findings of collaterals, which may recruit portal volume flow to the side that was deprived of portal flow during the procedure, a correct estimation the perfused volume at day 7 is difficult. (B) Pressure gradient between in the main portal vein and the inferior vena cava was measured intraoperatively by using 18-gauge intraportal needles. The results indicate an acute portal hypertension as a result of both of the devascularization procedures, PVL and PVL+HVL. In both procedures the porto-caval gradients return to normal after 7 days. (C) Aspartate transferase (AST) levels before and after the procedure at day 0, at day 1, and every other day until day 7. There is no significant increase on day 0 after PVL; there was an increase after PVL+HVL ($P < .001$), but no difference between groups. (D) Alanine transferase (ALT) levels. (E) Total bilirubin (tbili) levels. (F) International normalized ratio (INR) levels. There was no significant difference between time points or group for ALT, AST, tbili, or INR. For all statistics in this figure, Friedman test for repeated measures of nonparametric data was used. Kruskal-Wallis test was used for comparison of groups.

Recently, improvements in outcomes have been described, both in highly selected patient populations[23] and in prospective series with more restrictive inclusion criteria.[24] There is accumulating evidence, however, that ALPPS induces a large volume of immature (hypertrophied) liver tissue in a short period that is voluminous but not fully functional and may put patients at risk of posthepatectomy liver failure more often than predicted by the large increase in parenchymal liver volume.[25–29] In contrast, there is now preliminary evidence in 3 patients that simultaneous double embolization in humans does not induce an incongruence in function versus volume.[12] It is an interesting finding that rapid hypertrophy comparable in magnitude to ALPPS does not necessarily appear to result in a functional deficit. The understanding of the physiologic elements associated with rapid hypertrophy remains an important task to clarify, and specifically whether the circulating growth factors like interleukin-6 and tumor necrosis factor α, the acute portal hypertension, or the difference in the extent of abrogation of collaterals ultimately explain the increase in volume and also the occurrence of the functional deficit after classic ALPPS and how functionality can be maintained in rapid hypertrophy. This large animal model confirms that rapid volume increase occurs after double ligation and will allow the ability to study questions of volume and function by comparing PVL, the ALPPS model, and double ligation.

There is evidence that the number of collaterals negatively correlates with the degree of hypertrophy in PVL and ALPPS.[7] In addition, there are case reports of failed hypertrophy as a result of developing porto-portal collaterals after portal vein embolization (PVE) in humans.[30] There also is the presumption that PVE is more effective than PVL in humans because of decreased collateralization in general when the portal venous space is filled with glue.[31] Going back almost 100 years, Peyton Rous[32] reported collaterals to the portal vein–deprived liver in the first paper on experimental portal vein ligation in 1921.[32] He noticed that “The rapidity and completeness of the atrophy is in our experiments proportionate

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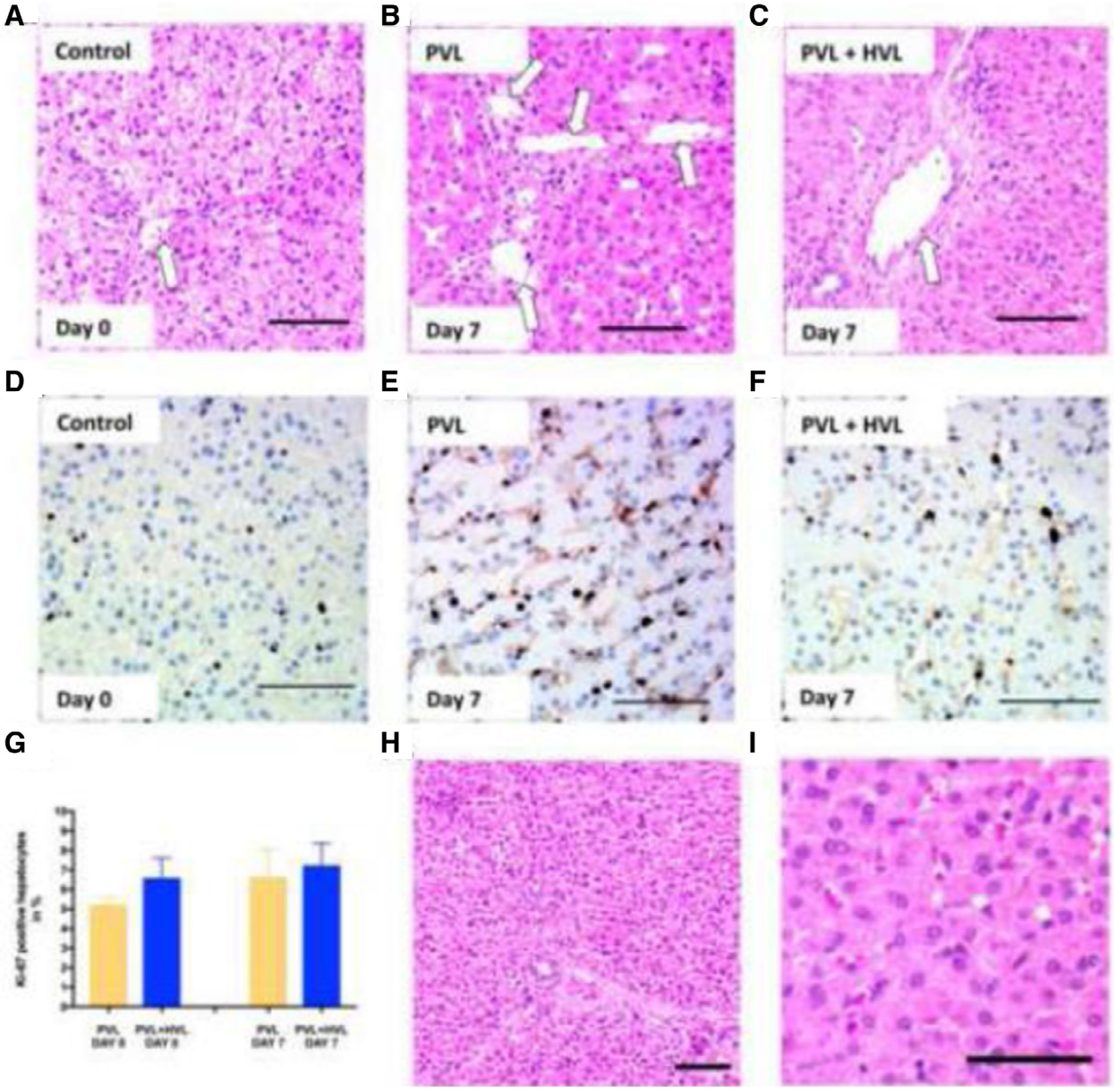

Fig. 6. Histologic examination 7 days after portal vein ligation (PVL) and PVL plus hepatic vein ligation (PVL+HVL). (A) Normal portal triad of a pig liver at day 0 before operation shows normal sizes of the portal vein (arrow), artery, and bile duct (×200 magnification). (B) Portal triad 7 days after PVL shows dilated portal veins (arrows) with normal diameters of the bile ducts and artery (×200 magnification). (C) Portal triad 7 days after PVL+HVL shows a dilated portal vein (arrow) (×200 magnification). (D) Ki-67 immunohistochemistry of a normal pig liver at day 0 before operation with staining of hepatocytes (arrow), occasional Kupffer cells (short arrow) (×400 magnification). (E) Ki-67 immunohistochemistry 7 days after PVL with staining of hepatocytes (arrow), occasional Kupffer (short arrow), and endothelial cells (thin arrow) (×400 magnification). (F) Ki-67 immunohistochemistry 7 days after PVL+HVL with a very similar appearance, staining of hepatocytes (arrow), occasional Kupffer (short arrow), and endothelial cells (thin arrow) (×400 magnification). (G) Quantitative evaluation of Ki-67 staining between PVL and PVL+HVL. No difference was detected. Friedman test for repeated measures of nonparametric data was used. Kruskal-Wallis test was used for comparison of groups. (H) No evidence was found of necrosis or degeneration of histologic architecture in the lobe deprived of the portal vein and hepatic vein 7 days after PVL+HVL (×100 magnification). (I) No evidence was found of necrosis, cytoplasmic degeneration, or inflammation 7 days after PVL+HVL with greater magnification (×600). Each bar in the lower right corner represents 100 microns, which was calibrated according to magnification using ImageJ.

to the number of these little collaterals. Their influence may be directly seen where they enter the liver."[32]

In the epoxy casts, a decrease and not a complete absence of collaterals in the double ligation model was found. Both models induced acute portal hypertension, and in both models, gradients normalized 1 week after ligation. It should be emphasized that hepatic veins, although occluded according to ultrasound imaging immediately after the performance of PVL+ HVL, had partially recanalized at day 7. These observations warrant future studies on the longitudinal hemodynamics of collateral flow along the time axis.

The histologic findings of this study and the comparable Ki-67 proliferation rates of hepatocytes are different from our findings in the ALPPS model in pigs[7] and suggest that the process of hepatocyte proliferation in PLV+HVL in pigs is terminated before day 7. Evaluations at earlier time points should address this in the future.

A methodologic limitation of this study is the lack of cross-sectional imaging in our large animal facility to allow a detailed, chronologic assessment of the changes in liver volume and possibly also an interrogation of in vivo inflow kinetics using contrast agents. Also, an assessment of liver function by regional liver function tests using hepatobiliary iminodiacetic acid scanning was not

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yet possible in this study owing to the absence of a gamma camera or single photon emission computed tomography for large animals. A combined assessment of volume and function would greatly enhance the understanding of the different types of liver hypertrophy and their functionality. Differently from the human studies on double embolization, this large animal model allows the study of the vascular anatomy after PVL and double ligation of portal and hepatic veins by performing epoxy vascular casts. These casts provide a static snapshot of anatomic detail of the collaterals that have developed; this technology allows us to study the development of collaterals that have not been reported by any other study so far.

Another limitation is that PVL+HVL is compared with PVL alone and not directly also to ALPPS or PVE. In a large animal model, the three Rs (reduce, refine, replace) of laboratory animal science led us to not repeat the previously published ALPPS series again but to compare the new results with PVL+HVL with data published about ALPPS in the pig.[7] The allocation into 2 groups, PVL versus HVL+PLV, is a simple study design. The power analysis for sample size was performed based on data from our previously published study of PVL versus ALPPS. The random allocation resulted in an asymmetric allocation of 8 vs 6 animals, which is methodologically unavoidable. In the future, PVE in the pig and also hepatic vein embolization in this laboratory will be tested, but this concept will require a new experimental setup with fluoroscopy and involvement of interventional radiologists.

In conclusion, this preclinical study reports on the feasibility and tolerability of simultaneous unilateral occlusion of hepatic and portal veins to induce rapid liver regeneration in the pig model. It may be the case that the double venous ligation or embolization technique in humans allows for a safer acceleration of hypertrophy than has been found with ALPPS so far. Double ligation and embolization should be evaluated further with careful analyses of liver function to evaluate this potential alternative to ALPPS in regenerative liver surgery.

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