Selective Portal Vein Embolization and Ligation Trigger Different Regenerative Responses in the Rat Liver

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Two strategies are clinically available to induce selective hypertrophy of the liver: portal vein embolization (PVE) and portal vein ligation (PVL). The aim of this study was to compare the impact of PVE and PVL on liver regeneration. Rats were subjected to 70% PVL, 70% PVE, 70% partial hepatectomy (PH) (positive control), or sham operation (negative control). PVL and PVE of liver segments were validated by portography and histology, demonstrating obstruction of the involved portal branches. Liver weight and markers of regeneration were assessed at 24, 48, and 72 hours, and 7 days after surgery (n = 5). Sinusoidal perfusion was examined by intravital microscopy. The weight of the regenerating liver segments increased continuously in all groups, with the highest weight gain after PH, which also disclosed the strongest proliferative activity. In Ki-67 and PCNA stainings, hepatocyte proliferation after PVL was more pronounced than after PVE ($P = 0.01$). Volumetric blood flow and functional sinusoidal density were lower after PVE than after PVL ($P = 0.006$, $P = 0.02$, respectively). The accumulation of Kupffer cells 24 hours after the intervention was highest after PH. Transcript levels of cytokines (interleukin-1β, tumor necrosis factor-α, interleukin-6) peaked at 24 hours and were highest after PH. The embolized part of the liver after PVE showed prominent foreign body reaction in the portal triad with accumulation of macrophages. *Conclusion:* PVL is superior to PVE in inducing a regenerative response of the remnant liver. The impairment of liver regeneration after PVE may be a consequence of macrophage trapping in the occluded segment due to a foreign body reaction. Lower blood flow and lower accumulation of macrophages, particularly Kupffer cells, in the regenerating part of the liver likewise causes impaired liver regeneration after PVE. (HEPATOLOGY 2008; 47:1615-1623.)

Liver resection is the only chance of cure in many patients with primary or secondary liver tumors.[1] One limiting factor for success is the size and function of the remnant liver left after surgery.[1-2] Owing to the unique and versatile ability of the liver to regenerate, a number of strategies have been developed to increase the volume of a potential future remnant liver. As early as a century ago, experiments in rabbits showed that selective occlusion of a large branch of the portal vein causes atrophy of the ipsilateral lobe and hypertrophy of the contralateral liver lobe.[3] In the late 1980s, Makuuchi et al.[4] first applied selective occlusion of a branch of the portal vein to induce hypertrophy of the future remnant liver in patients requiring major hepatectomy. This strategy is currently used in many centers worldwide to extend the limit of liver resection.[1]

Selective occlusion of tributaries of the portal vein can be achieved in patients by portal vein embolization (PVE), usually performed percutaneously with microspheres, cyanoacrylate, fibrin glue, ethanol, or by portal vein ligation (PVL) performed during open or laparoscopic surgery. Although many clinicians believe that PVE is superior to PVL,[5] convincing data is lacking. One retrospective study in patients undergoing major hepatectomy suggested better regenerative ability of the liver after PVE rather than after PVL.[5] Another recent prospective

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; FSD, functional sinusoidal density; HPF, high-power field; IL-6, interleukin 6; IL-1β, interleukin β; PCNA, proliferating cell nuclear antigen; PH, partial hepatectomy; PVE, portal vein embolization; PVL, portal vein ligation; TNF-α, tumor necrosis factor α; V_{RBC}, red blood cell velocity; VBF, volumetric blood flow.

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study in patients undergoing preoperative PVE prior to a hemihepatectomy failed to show any clinical advantages.[6] A subsequent study from the same group suggested better regenerative ability after PVL than after PVE.[7] These controversies are further enhanced by the lack of validated animal models of PVE. Likewise, the regenerative response differs significantly considering that PVL results in the complete absence of flow proximally to a well-defined territory of the liver, whereas PVE causes impairment in flow by foreign bodies located peripherally, usually in the presinusoidal veins.

Therefore, we compared the impact of PVE and PVL on liver regeneration of the contralateral segments and atrophy of the ipsilateral segments. We first validated comparable models of PVL and PVE, and used an established model of partial hepatectomy (PH) as a positive control. We also explored putative mechanisms of regeneration, which may explain the different response in inducing hepatocyte proliferation.

Materials and Methods

Animals. The experiments were performed on male Wistar rats weighing 250-280 g (Harlan, Horst, The Netherlands). The animals were kept in the animal facility of the University Hospital Zurich with access to standardized chow and water ad libitum. All procedures were approved by the Veterinary Office of the Canton Zurich and were performed between 8 AM and 12 AM, in compliance with institutional animal care guidelines. Group size was n = 5 per each study group unless otherwise indicated.

Operative Procedure. Four groups of experiments were used which comprise a model of 70% PH, a selective PVL, selective PVE, or sham operation at 4 different times. The rats were anesthetized with inhalation of isoflurane/O_2 (Halocarbon Laboratories, River Edge, NJ), and a constant gas mixture was maintained with a vaporizer system (Provet, Basel, Switzerland). After a midline laparotomy, the liver was freed from its ligaments. Buprenorphin (0.1 mg/kg body weight) was applied intraperitoneally during anesthesia at the end of the operation and repeated subcutaneously 12 hours later.

Sham-operated animals were closed again by a double running suture.

Partial Hepatectomy. A model of 70% PH was performed according to a modification[8,9] of the standard method described by Higgins and Anderson in 1931.[10] Briefly, after a midline laparotomy, the liver was freed from its ligaments. Subsequently, we performed the ligation of the whole left lateral lobe, right hepatic lobe (divided by a horizontal fissure into superior and inferior right lobe), and the caudate lobe (divided in anterior and posterior caudate lobe) with 6-0 silk followed by resection of ligated liver lobes.

Portal Vein Ligation. Selective PVL was performed on quadrant lobe, left lobe, upper right lobe, and lower right lobe. After careful dissection of the hepatic artery, the corresponding portal veins of the liver were ligated with 7-0 Fumalen. The portography was first performed before the PVL to visualize the liver anatomy, and then was repeated after the selective PVL to demonstrate portal occlusion of appropriate liver segments.

Portal Vein Embolization. For PVE, the cental portal vein was punctured with a 20-gauge needle (Insyte; BD, Franklin Lakes, NJ). The needle was connected with a 3-way stopcock and a 1 mL syringe filled with 0.9% physiological saline solution to prevent air bubble intrusion. The syringe and the catheter were fixed with 6-0 silk. Afterward, we performed portography of the liver by injecting 1 mL of Telebrix (1:4 diluted with 0.9% NaCl: 0.25 mL Telebrix + 0.75 mL 0.9% NaCl) into the portal vein. As a next step, 30% of the portal vein was occluded with the vascular clamp (Aesculap, Ref. FE710K). Then 1 mL of Embosphere (Ref. S220GH) diluted 1:10 in 0.9% NaCl was injected. Embosphere is an acrylic copolymer trisacryl, cross-linked with gelatin, that allows a precise match of sphere and vessel lumen diameters. This material is nonaggregating and is able to increase its volume due to its hydrophilic surface. After the successful embolization, the catheter was flushed with 0.2 mL 0.9% physiological saline solution. Portography was repeated to demonstrate occlusion of the appropriate liver segments. The hepatic artery remained patent in both the PVL and PVE groups. At the end of surgery, the abdomen was closed by 4-0 Vicryl double layer running suture.

In Vivo Fluorescence Microscopy and Analysis of Microcirculation. Intravital microscopy was performed as described.[11] Functional sinusoidal density (FSD) was defined as the total length of all perfused sinusoids per observation area (cm/cm^2).[12] The red blood cell (RBC) velocity (V_{RBC}) and the sinusoidal diameter were measured in 10 sinusoids in the midzonal region. The volumetric blood flow (VBF) in each sinusoid was calculated from the velocity of RBCs and the cross-sectional surface area ($\pi \times r^2$) according to the following equation[13]: VBF = $V_{RBC} \times$ cross-sectional surface area. FSD and the sinusoidal diameter were assessed by a computer-assisted image analysis software system (CapImage; Zeintl Software, Heidelberg, Germany).

Analysis of Portal Flow. The portal flow was measured in the main trunk of the portal vein before its division into the liver (Doppler Transonic Systems Inc., Altron, Germany).

Liver Weight. After PH and harvesting (PVL and PVE groups) the weight of the regenerating, the resected, and the atrophied lobes was measured using a laboratory micro scale (Sartorius AG, Goettingen, Germany).

AST and ALT Levels. The blood samples obtained from the inferior vena cava at different time points of liver regeneration were immediately centrifuged at 2000*g* for 6 minutes. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured using a serum multiple biochemical analyzer (Ektachem DTSCII, Rochester, NY).

Histological Examination. The liver tissues were immersion fixed in 4% formaldehyde, embedded, sectioned, and stained with hematoxylin-eosin (H-E). The liver sections were immunostained for proliferating cell nuclear antigen (PCNA) (fluorescein isothiocyanate–labeled monoclonal mouse clone PC10, Abcam, Cambridge, UK) and Ki-67 (monoclonal rabbit clone SP6, NeoMarkers; Lab Vision Corp., Fremont, CA) using the Ventana Discovery automated staining system with iView DAB kit (Ventana Medical Systems Inc., Tucson, AZ). Sections were also stained with the monoclonal ED1 antibody (CD68 Serotec/Carnon, Germany) and α-SMA (MU128-CU; Biogenex, San Ramon, CA), using a streptavidin-biotin immunoperoxidase method.

All immunostains were counterstained with hematoxylin. Neutrophilic granulocytes were stained with the AS-D chloroacetate esterase (CAE) technique. The number of Ki-67–positive and PCNA-positive hepatocytes were determined in 10 random visual fields (200×), and $ED1^+$ monocytes/macrophages and neutrophilic granulocytes were determined in 10 random high-power fields (HPFs) (400×). The necrotic areas after PVE or PVL were quantified in 10 visual fields (100×) using the Carl Zeiss AxioVision 4 LE program (Carl Zeiss, Jena, Germany).

All histological analyses were performed in a blinded fashion with respect to the experimental groups.

Quantitative Real-Time Polymerase Chain Reaction. The total RNA was extracted from 50 mg liver tissue using TRIzol reagent (Invitrogen, Paisley, UK). Five micrograms RNA were reverse-transcribed using the Thermoscript RT-PCR kit (Invitrogen, Basel, Switzerland), yielding the complementary DNA template. The quantitative real-time polymerase chain reaction (PCR) amplification and the data analysis were performed using an ABI-Prism 7000 Sequence Detector system. TaqMan gene expression assays (PE Applied Biosystems, Rotkreuz, Switzerland) for IL-1β (Rn00580432_m1), IL-6 (Rn00561420_m1), and TNF-α (Rn99999017_m1) were used to quantify the messenger RNA (mRNA) expression of the respective genes. The mRNA expression levels for each sample were normalized to 18*S* RNA. The results gained represent fold induction versus baseline levels in control rats.

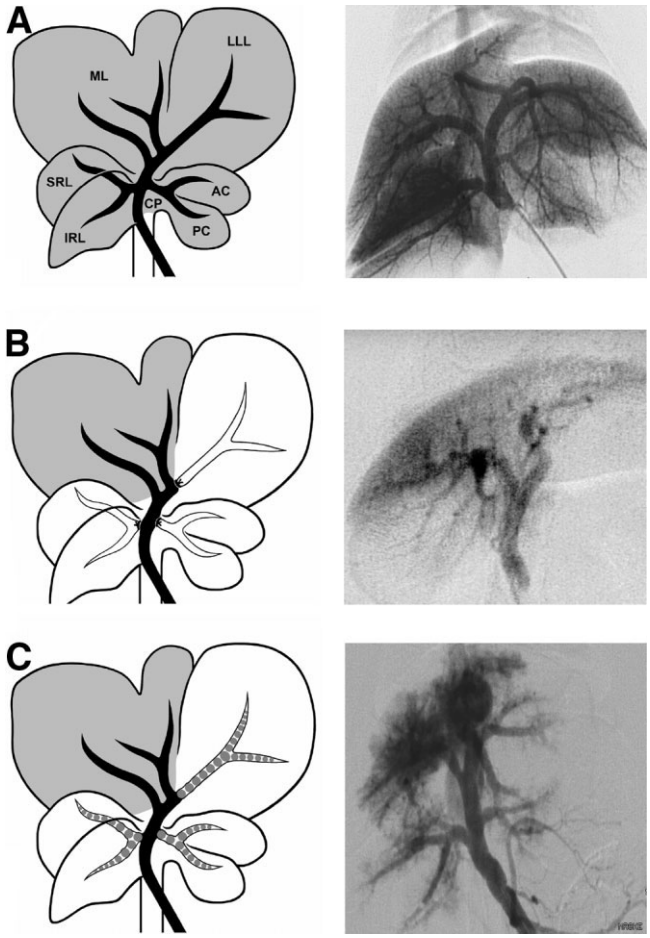

Fig. 1. The rat liver before and after PVL and PVE. (A) Schematic anatomy of the rat liver lobes and portal vein (left) and a representative portography of the liver (right). Rat liver lobes: median lobe (ML), the left lateral lobe (LLL), the right lobe divided into superior right lobe (SRL) and inferior right lobe (IRL), and caudate lobe formed by the caudate process (CP) and Spiegel lobe with anterior (AC) and posterior (PC) portion. (B) Schematic presentation of PVL and portography after PVL. (C) Schematic presentation of PVE and portography after PVE.

Statistics. All data are expressed as means ± standard deviation (SD). Differences between the groups were assessed by 1-way or 2-way analysis of variance (ANOVA, Bonferroni post-test), using an appropriate post-hoc comparison test, including Newman-Keuls probabilities to compensate for multiple comparisons. Ordinal variables were compared with the Mann-Whitney test. A significant difference was assumed when P was smaller than 0.05. Statistics were performed using the software package GraphPad 4.0 (GraphPad Software Inc., San Diego, CA) and SPSS 12.0.1 (SPSS Inc., Chicago, IL).

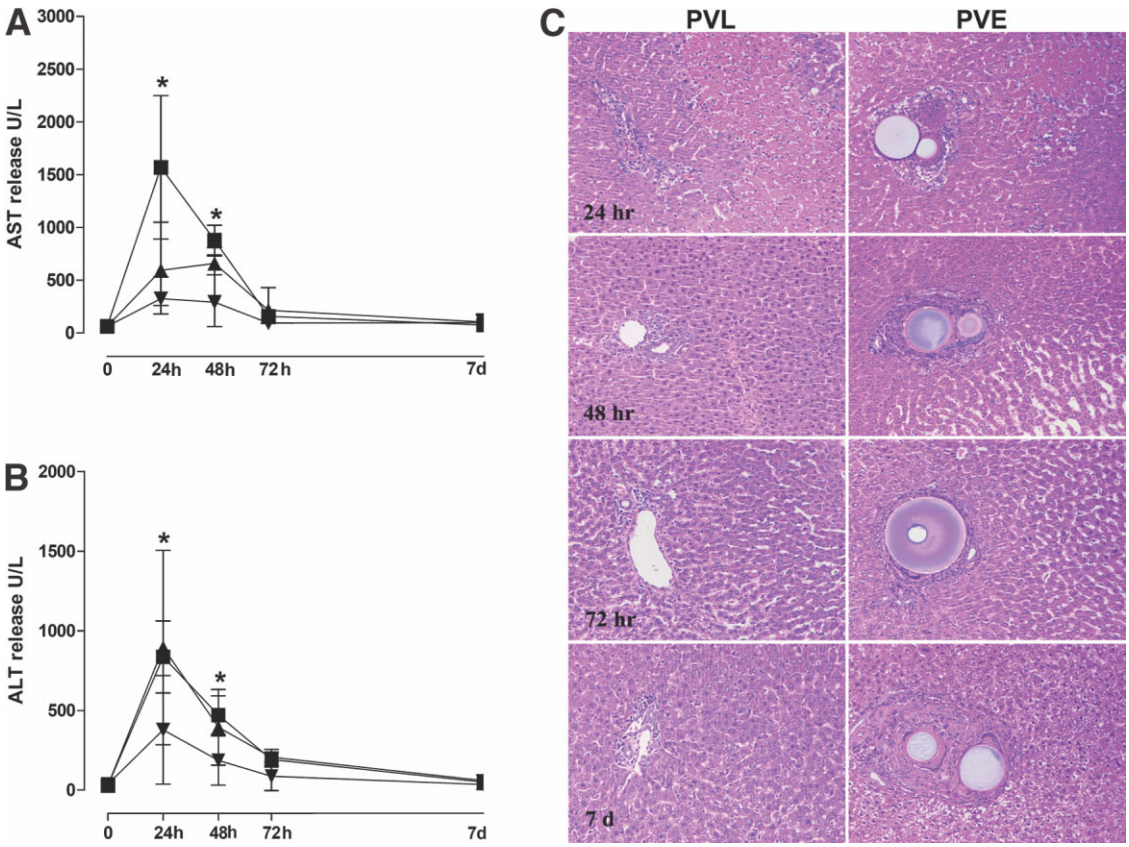

Fig. 2. Quantification of hepatocellular injury after different surgical interventions. (A) AST and (B) ALT release in all time points. Symbols denote PH (■), PVL (▲), and PVE (▼). (C) Histological analysis of the occluded liver lobes after PVL and PVE at 24, 48, and 72 hours and 7 days after the intervention. There was a significant difference in AST release between PH and PVL at 24 hours after surgery ($P < 0.001$) and between PH and PVE at 24 and 48 hours ($P < 0.001$ and $P < 0.05$, respectively). The ALT release shows a significant difference between PH and PVE at 24 and 48 hours ($P < 0.01$ and $P < 0.05$, respectively). N = 5 for all subsequent experiments except for Fig. 8. Values are means ± SD. Two-way ANOVA indicated that the curves were significantly different for AST ($P = 0.0005$) and for ALT ($P = 0.0056$) using the Bonferroni post-test.

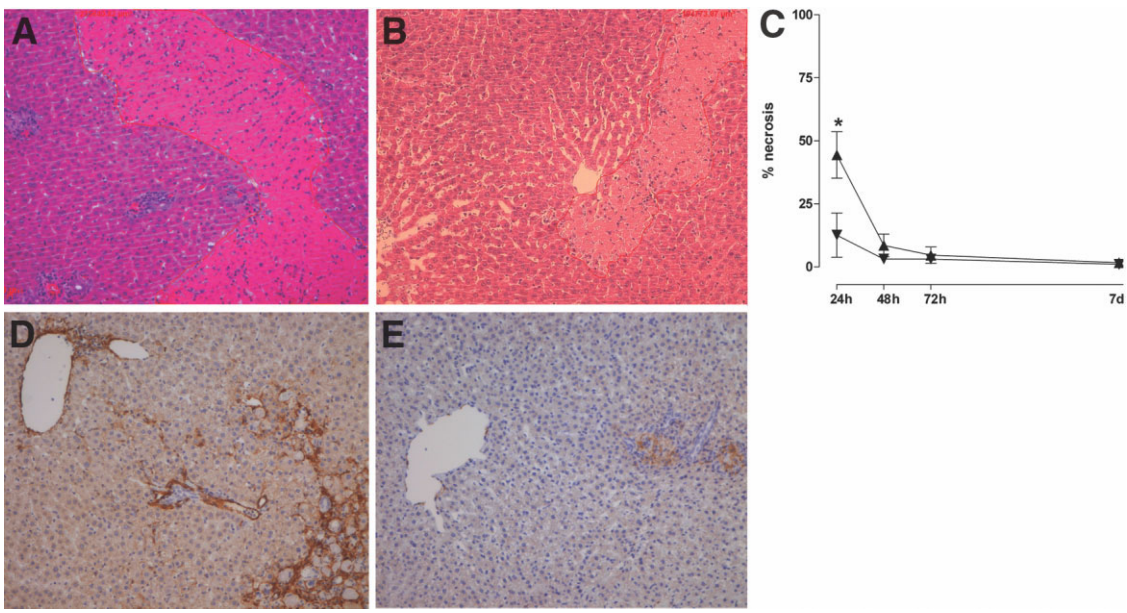

Fig. 3. Perivascular necrosis in liver lobes after (A) PVL and (B) PVE at the 24 hour time point. Morphometric analysis of the necrotic areas per visual field (100×) in all time points (C) show a significant difference at 24 hours ($P < 0.001$). Values are means ± SD. Two-way ANOVA indicated that the curves were significantly different for area of necrosis ($P = 0.0002$) with the Bonferroni post-test. Analysis of the atrophic tissue 7 days after (D) PVL and (E) PVE for myofibroblasts (α-SMA staining).

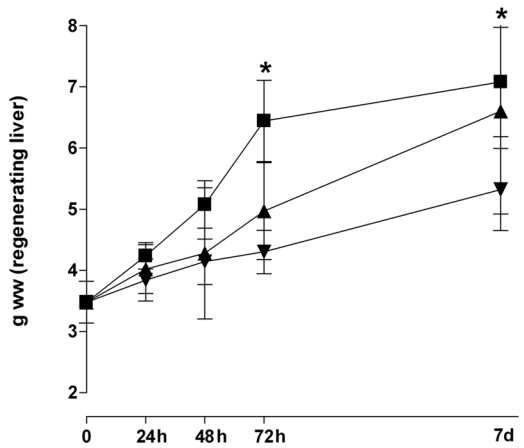

Fig. 4. Increase of liver weight of regenerating lobes after PH, PVL, and PVE. Liver weight after various surgical procedures are presented at 4 different time points. Symbols denote PH (■), PVL (▲), and PVE (▼). The increase of liver weight at 72 hours and at 7 days in the PVE group was significantly different to the PH group ($P < 0.01$ and $P < 0.05$, respectively). Values are means ± SD. Two-way ANOVA indicated that the curves were significantly different when comparing liver weights ($P = 0.002$) using the Bonferroni post-test.

Results

Are the Models of PVE, PVL, and Hepatectomy Comparable? First, we performed experiments to secure the effectiveness of the different strategies used to trigger a proliferative response. The model of PH is well established and consists of a 70% removal of the liver. The weight of the resected part of the liver compared to the total liver weight was 7.4 ± 0.7 g to 10.9 ± 0.5 g (n = 5) (68%). Both PVL and PVE were performed in the same liver segments that were resected for PH. The model of PVL and PVE consisted of the ligation or embolization of the same branch of the portal vein as used in the model of PH. We performed portography before PVL and PVE to document the liver vascular anatomy and afterward to record the occluded territory of the portal system, and the perfusion of the remnant liver (Fig. 1). Comparable territories of the liver were excluded from perfusion using either PVE or PVL, and we concluded that valid comparisons can be drawn from these models.

Do PVL or PVE Cause Liver Injury? Next, we tested whether PVE or PVL caused liver injury. We measured serum aminotransferase (AST and ALT) levels as established markers of hepatocyte injury and performed histological examination of the occluded part of the liver. Both PVE and PVL caused a comparable increase in AST and ALT levels, which were lower than after PH (Fig. 2).

Histological analysis after PVL or PVE revealed areas of necrosis in the occluded part of the liver, which were significantly larger after PVL than after PVE at 24 hours after surgery (44.4 ± 9.2% versus 12.6 ± 8.8%, $P < 0.001$) (Fig. 3). When tissue was analyzed 7 days after PVL and PVE (Fig. 3), the areas of necrosis appeared resolved. Consistent with the observation of centrolobular necrosis after PVL, we also found centrolobular accumulation of myofibroblasts (α-SMA staining). In contrast, myofibroblast accumulation was found around the occluded portal veins after PVE. The presence of fibrosis was tested by Sirius red staining of collagen, which showed no substantial fibrosis in the occluded liver segments after PVL and PVE.

What is the Impact of PH, PVE, and PVL on Liver Regeneration? To determine the effects of PH, PVE, and

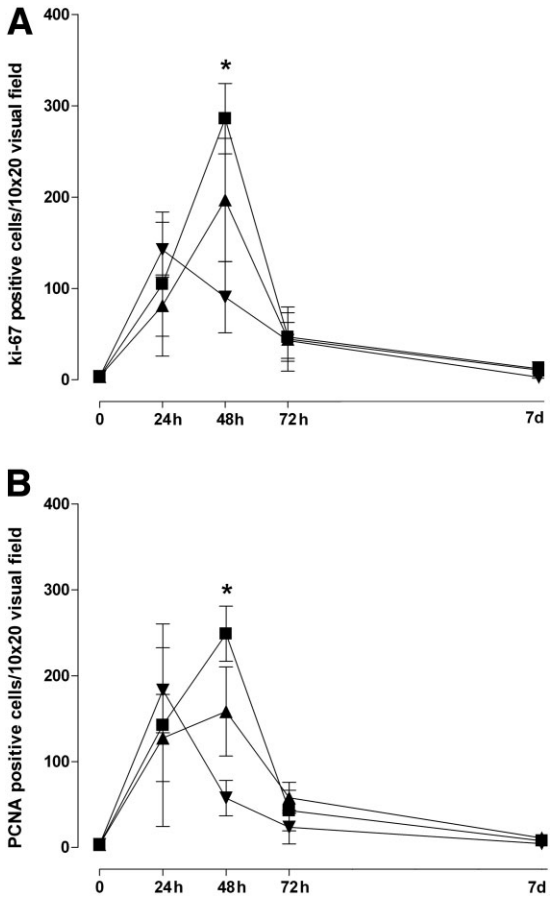

Fig. 5. Regeneration of remnant livers after different surgical interventions. Livers were analyzed for (A) Ki-67–positive and (B) PCNA-positive hepatocytes after PH (■), PVL (▲), and PVE (▼) at all different time points. The number of positive hepatocytes per visual field (200×) is presented. The increase of Ki-67–positive cells show significant differences: PH versus PVL ($P < 0.01$), PH versus PVE ($P < 0.001$), and PVL versus PVE ($P < 0.01$) at 48 hours after surgery. Values are means ± SD. Two-way ANOVA indicated that the curves were significantly different for increase of Ki-67–positive cells after different interventions ($P = 0.015$) using a Bonferroni post-test. PCNA also showed significant differences: PH versus PVL ($P < 0.05$), PH versus PVE ($P < 0.001$), and PVL versus PVE ($P < 0.05$) at 48 hours after surgery. Values are means ± SD. Two-way ANOVA indicated that the curves were significantly different for increase PCNA-positive cells after different interventions ($P = 0.036$) using a Bonferroni post-test.

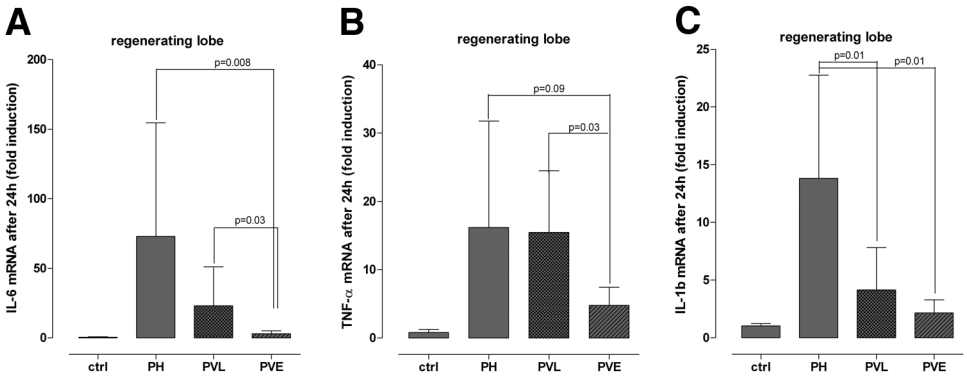

Fig. 6. Effect of different surgical interventions on expression of inflammatory mediators in regenerating liver tissue at 24 hours after PH, PVL, and PVE. The mRNA levels coding for IL-6, TNF-α, and IL-1β were determined by RT-PCR and given as fold induction relative to sham-operated livers. Statistically significant differences are indicated by bars (means $\pm$ SD) and *P* values (Mann-Whitney test with 2-tailed *P* value).

PVL on contralateral liver regeneration, we measured the liver weight and markers of hepatocyte proliferation. PH induced the highest response with an increase of the remnant liver from baseline of 3.5 g ($\pm$ 0.3) to 7.1 g ($\pm$ 0.9) by postoperative day 7 (Fig. 4). PVL exhibited a delayed growth but reached a similar weight 7 days after ligation. In contrast, PVE induced a blunted regenerative response reaching a maximal size of 5.3 g ($\pm$ 0.7) at 7 days after embolization.

To further characterize the regenerative response, we assessed Ki-67 and PCNA staining, which are 2 nuclear antigens associated with proliferation at all time points after surgery (Fig. 5). As expected, both markers were expressed in numerous hepatocytes in the PH group. PVL and PVE exhibited reduced proliferative activities at 48 hours, which was consistent with the liver weights. Both Ki-67 and PCNA labeling indices were lower in the PVE group (Fig. 5).

What Factor Might Explain the Different Regenerative Response? We next focused on possible mechanisms explaining the different regenerative response. Although the observation of the highest response associated with PH was expected, the superior response of PVL over PVE was not. Our first line of investigation focused on the release of established mediators of liver regeneration including TNF-α, interleukin IL-1β, and IL-6. These cytokines are released from nonparenchymal liver cells (that is, Kupffer cells) after hepatectomy[14] and promote hepatocyte to DNA synthesis and hepatocyte proliferation. Evidence has been provided that activated Kupffer cells produce TNF-α which in turn up-regulates the expression of IL-6. TNF-α and IL-6 activate neighboring hepatocytes[15] to move from the G_0 phase to the proliferative cycle.[15-19] These growth cytokines were highly up-regulated in the regenerating lobe 24 hours after PH compared to sham-operated animals (Fig. 6). We observed a significant increase in growth cytokines after PVL, although less than after PH. In contrast, we failed to demonstrate a significant cytokine response after PVE. These data are consistent with the regenerative response observed in the previous set of experiments. In the second line we focused on the activation of growth factor mediated pathways during liver regeneration and measured vascular endothelial growth factor (VEGF) which binds to endothelial cells, subsequently triggering the release of hepatocyte growth factor (HGF) from stellate cells. There were no statistically significant differences between the experimental groups at all different time points (Supplementary Figs. 1, 2).

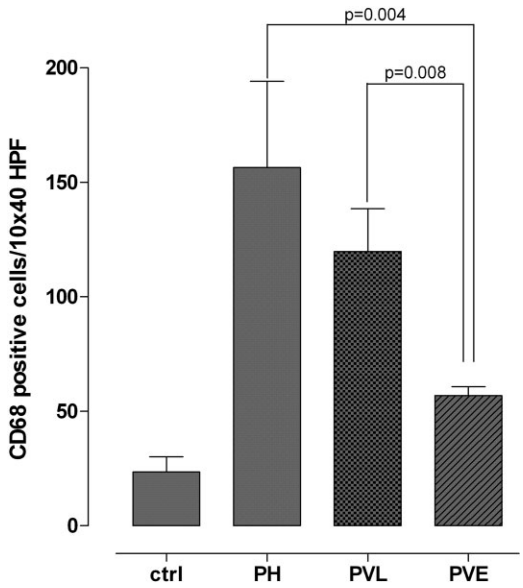

Fig. 7. Kupffer cell/macrophage accumulation in regenerating liver lobes. Immunohistochemistry of liver sections stained with ED1 (CD68) as a marker for monocytes and macrophages at 24 hours. Positive cells were counted and expressed as mean number of macrophages per high-power field (400$\times$). Values are means $\pm$ SD. Mann-Whitney test with 2-tailed *P* value.

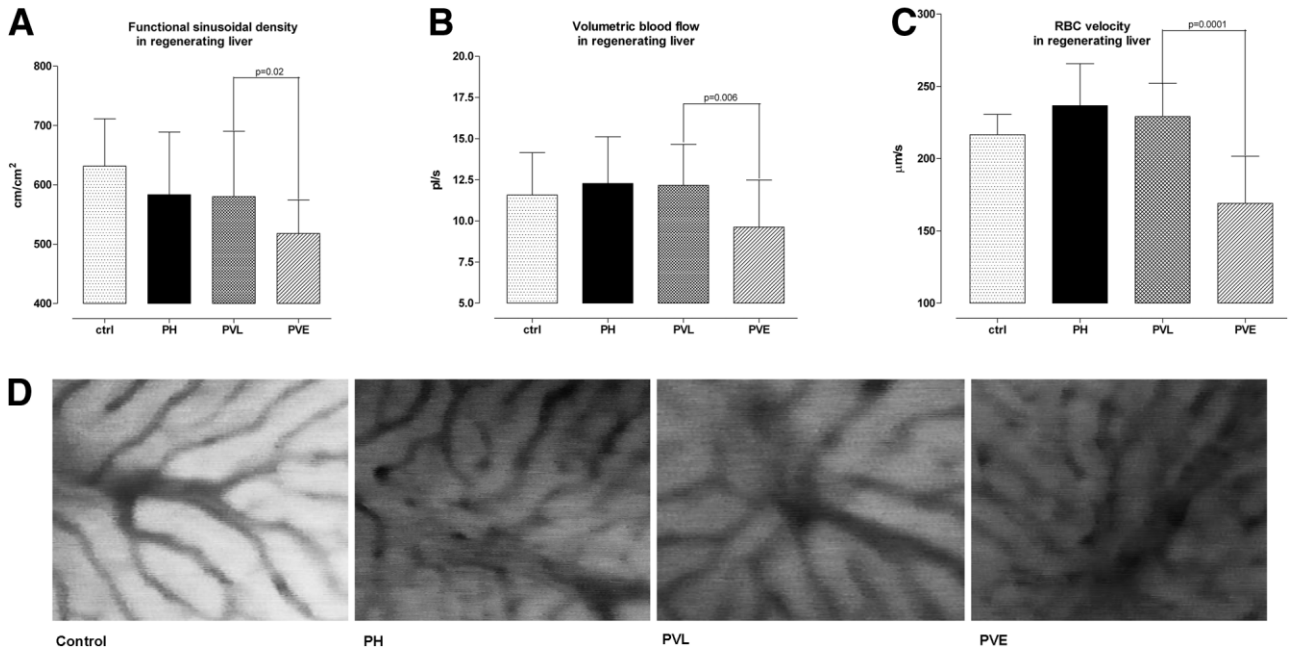

Fig. 8. Hepatic microcirculation of regenerating livers using in vivo fluorescence microscopy. Quantitative analysis of (A) functional sinusoidal density, (B) volumetric blood flow, and (C) RBC velocity in regenerating liver lobes at 24 hours after different surgical interventions. (D) Representative intravital fluorescence microscopy images displaying the functional sinusoidal density in control liver and after PH, PVL, and PVE. N = 3 per group. Statistical significance indicated by bars (means ± SD) and the respective Mann-Whitney test with 2-tailed *P* value.

Considering that the putative primary sites of cytokine production after hepatectomy are the Kupffer cells, we next determined the number of Kupffer cells in the regenerating lobe, by using immunohistochemistry. In sham control animals, 23 ± 6.7 positive cells/HPF were detected throughout the liver. PH caused a 6-fold increase in the density of Kupffer cells 24 hours after surgery (156 ± 37.7 positive cells/HPF) (Fig. 7). PVL resulted in a slightly lower number of $ED1^+$-positive cells in the regenerating lobe (120 ± 18.8 positive cells/HPF), whereas PVE induced a significant but limited (3× increase) response with 57 ± 3.8 positive cells/HPF. This data is in line with the previously observed regenerative response and growth cytokine release.

Do PVE and PVL Result in Similar Degree of Changes in Portal and Sinusoidal Flow? One explanation for the differences in the regenerative response between PVL and PVE might be related to a difference in the ability to block portal flow in the ipsilateral lobe and to induce higher portal pressure in the contralateral lobe. We assessed sinusoidal perfusion 24 hours after surgery using intravital microscopy. Functional sinusoidal density, volumetric blood flow, and erythrocyte velocity (V_{RBC}) were lower in regenerating livers after PVE in comparison to PVL and PH (Fig. 8, Table 1). To assess whether these changes correlate with portal flow in the PVL model, we measured portal flow using a Doppler transonic system 24 hours after selective portal

Table 1. Values of Functional Sinusoidal Density, Volumetric Blood Flow and RBC Velocity in Regenerating Liver Lobes Using In Vivo Fluorescence Microscopy, at 24 Hours After Sham Laparotomy, PH, PVL, and PVE

Measurement	control	PH	PVL	PVE
Functional sinusoidal density (cm/cm²)				
Mean	631.4	583.6	580.2	518.0
Standard Deviation	79.94	105.5	110.3	56.71
95% CI of mean	601.6	544.2	539.1	496.9
Volumetric blood flow (pl/second)				
Mean	11.58	12.28	12.16	9.627
Standard Deviation	2.576	2.835	2.497	2.860
95% CI of mean	10.62	11.22	11.23	8.559
RBC velocity (µm/second)				
Mean	216.6	236.7	229.2	169.0
Standard Deviation	13.97	29.00	22.94	32.61
95% CI of mean	211.4	225.9	220.6	156.9

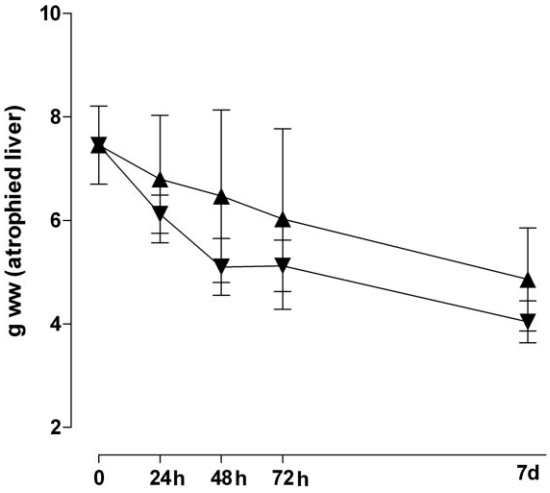

Fig. 9. Influence of PVL and PVE on liver atrophy. Weight of the ipsilateral lobes after various surgical procedures are presented at 4 different times. PVL = portal vein ligation (▲), PVE = portal vein embolization (▼). Values are means ± SD. ($P = 0.1260$, 1-way ANOVA).

vein occlusion. There were no statistically significant differences in the main portal flow between the PVL (18.0 ± 0.5) and PVE (15.5 ± 3.3) groups, whereas in the hepatectomized group the flow was higher (22.7 ± 3.0 mL/minute). This data suggests that the observed decrease in sinusoidal perfusion after PVE is related to "intraparenchymal" mechanisms, rather than different hemodynamic effects on the portal flow after PH, PVE, and PVL.

Do Changes in the Atrophied Liver Lobe Influence the Regenerative Response of the Contralateral Lobe? The striking difference between PVE and PVL in the regenerative response of the contralateral liver lobes led us to investigate changes in ipsilateral lobes. Both PVE and PVL lead to a comparable atrophy of the ispilateral liver segments (Fig. 9). Next, we looked at changes in H-E stained sections of the atrophied lobe.

The histological assessment of the PVE group demonstrated the presence of microspheres in small presinusoidal branches of the portal vein as sequels of the embolization (Fig. 10). Another striking finding was the presence of massive inflammatory reaction surrounding the microspheres. To further identify the infiltrating cell type, we performed specific staining for neutrophilic granulocytes and macrophages. The majority of the infiltrating cells were macrophages, with a very modest infiltration by neutrophilic granulocytes (Fig. 10).

Discussion

This study was designed to shed some new light on the ability of 2 widely used clinical strategies of selective portal vein occlusion to induce a regenerative response on the contralateral liver segments. After establishing reliable models of PVE and PVL in the rat, we found a significantly enhanced regenerative response after PVL, when compared to PVE. These unexpected findings seem related to a "foreign body reaction" with massive trapping of macrophages in the occluded part of the liver after PVE causing decreased accumulation of macrophages and release of growth cytokines in the contralateral (regenerative) segments. The additional impaired microcirculation observed after PVE in the regenerating liver appears to be rather a consequence of "intraparenchymal" mechanisms than changes in hemodynamics in the portal vein system.

A critical part of such studies is the establishment of reliable and relevant models. In the human situation, it is necessary to occlude more than 50% of the portal vein flow to induce a sustained regenerative response on the contralateral side. We chose in both PVE and PVL models a 70% occlusion of the portal supply to the liver in order to compare the results with the well-established model of 70% hepatectomy. The use of portography enabled us to convincingly identify the obstructed sectors of the portal vein. We used microspheres to cause distal PVE, a common modality used in patients worldwide. Other embolitic materials are also used occasionally for PVE such as fibrin glue, cyanoacrylate and ethiodized oil, gelatin, and absolute alcohol.[20] Because the regenerative response was impaired due to the massive foreign body

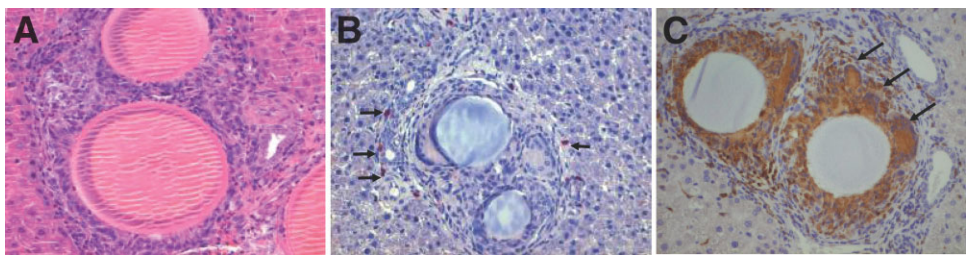

Fig. 10. Effect of injection of embolizing microspheres on granulocyte infiltration and accumulation of Kupffer cells in rat livers 24 hours after PVE in the atrophied lobe. (A) Embolized part of livers were processed for H-E staining. (B) Granulocytes were stained using alpha-naphthyl chloroacetate estase staining (see arrows). (C) Monocytes and macrophages, including Kupffer cells, were detected by immunohistochemistry using ED1/CD68 antibody.

reaction in the liver, it is possible that different responses may be observed with different embolizing materials.

The mechanism of regeneration identified in this study is novel and may have significant implications for the future. The data converge to a primarily massive and diffuse foreign body reaction around the microspheres after PVE. Macrophages rapidly accumulate in the occluded part of the liver, and may thereby not be available on the contralateral regenerating liver, blunting the initial proliferative response. The decreased amount of growth cytokines (TNF-α, IL-6, and IL-1β) is consistent with this observation. Another explanation could have been related to changes in the main portal flow influencing the regenerative response. Although we failed to show significant differences in the main portal flow among PVE and PVL experimental groups, we did observe impaired microcirculation in the regenerating liver in the PVE group, but not in the PVL group. These data suggest that this alteration in microcirculation is related to an "intraparenchymal" mechanism, possibly including the release of soluble factors from Kupffer cells.

The success of PVL was somewhat unexpected and may provide further incentive to use this strategy in patients. Interestingly, a recent study[7] suggested more efficient regeneration with selective PVL compared to PVE. This study was, however, retrospective and conducted in a relatively low number of patients, and therefore caution is still required in recommending this modality in the clinic. Nevertheless, the results of our study provide strong evidence for the efficacy of PVL in inducing contralateral hypertrophy of the liver. Accumulation of macrophages, the presence of growth factors, and markers of regeneration were all significantly documented in the contralateral segments. The values were close to the positive control of 70% PH.

The results may also have implications for patients with liver tumors, who have a high tumor load and insufficient normal liver to tolerate a major liver resection. We recently showed that occlusion by PVL did not result in enhanced tumor growth on the contralateral side.[21] The impact of PVE and PVL on tumor growth will need to be studied with caution to optimally use these strategies in patients. In these patients, portal occlusion is used prior to liver resection after inducing hypertrophy of the contralateral segment.

In conclusion, we demonstrated in a rat model that selective induction of liver hypertrophy is highly dependent on the type of intervention. The superiority of PVL may be related to a full regenerative response including accumulation of macrophages and the release of growth cytokines. Strategies to prevent the massive foreign body reaction associated with PVE through use of microspheres may permit improved regeneration through this modality, which is easily applicable in patients through a percutaneous approach. PVL requires surgery using either a laparoscopic or open approach. This study opens the door for a number of experimental and clinical studies, for example, in using different materials for PVE, and studying the effects of tumor growth while a part of the liver is regenerating.

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