Clinical and experimental studies on portal vein embolization / Diagnosis of hepatocellular adenoma and focal nodular hyperplasia
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Portal vein embolization induces more liver regeneration than portal vein ligation in a standardized rabbit model

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Abstract

Background: Portal vein ligation (PVL) and portal vein embolization (PVE) are used to induce hypertrophy of the future remnant liver before major liver resection. The aim of our study was to compare the hypertrophy response of the liver after PVL versus PVE in a rabbit model.

Materials and methods: Twenty rabbits were divided into an embolization group (n = 10) and a ligation group (n = 10). Both groups were divided in 2 subgroups of 5 rabbits that were humanely killed after days 7 and 14. The portal vein branches to the 3 cranial liver lobes (80% of the liver) were occluded. Regeneration of the caudal liver lobe was measured using volumetry based on computed tomography on days 3, 7, 10, and 14. Immunohistochemistry for Ki-67 and RAM11 was performed to quantify proliferating cells and macrophages. In addition, tissue tumor necrosis factor-α and interleukin-6 were assessed.

Results: The caudal liver volume increased over time in both groups (p < .001), but this increase was greater after PVE than after PVL (p = .001) with a mean degree of hypertrophy of 15% ± 4% and 20% ± 2%, respectively. When comparing the groups on the separate time points, a difference was found on days 10 and 14 (p = .008 and p = .016, respectively). These data were confirmed by Ki-67 staining, which showed a greater number of proliferating hepatocytes on day 7 after embolization (p = .016). Cytokine analysis of liver tissue did not show significant differences between the ligation and embolization groups on days 7 and 14.

Conclusion: PVE is superior to PVL in terms of the extent of the hypertrophy response in this rabbit model.
Introduction

Portal vein ligation (PVL) and portal vein embolization (PVE) are used to induce hypertrophy of the future remnant liver (FRL) before major liver resection\(^1,2\) in patients with an otherwise too small FRL.\(^3,4\) PVL is an invasive procedure in which the portal vein is ligated during laparotomy.\(^6\) PVE is a minimally invasive technique that can be performed percutaneously as well as during laparotomy.\(^2,7\) Using both methods, the portal blood flow is blocked, but only after PVE are the peripheral portal vessels occluded completely by the embolization material. It is important to use a portal vein occlusion technique that provides adequate hypertrophy of the nonembolized lobe in as short a time interval as possible, because of potential tumor progression after the procedure.\(^8-10\) Clinical and experimental studies show opposite results regarding which embolization technique leads to a greater regeneration response. One prospective study in patients concluded that PVE is superior to PVL in terms of volume gain, lesser time to hypertrophy, lesser hospital stay, and fewer adhesions during major hepatectomy.\(^11\) Another retrospective clinical study showed that PVL is as effective as PVE in inducing hypertrophy of the FRL.\(^12\) In both studies, however, the patient characteristics were not comparable and the methods used were not standardized and could have affected the outcomes.

An animal model is useful to study the effects of PVE and PVL in a standardized fashion. Two animal studies have compared the effect of PVL and PVE on the hypertrophy response; however, these studies showed conflicting results. Furrer et al.\(^13\) performed a study in rats and concluded that PVL is superior to PVE in inducing a regenerative response of the remnant liver, because the amount of proliferating hepatocytes was significantly greater in the PVL compared with the PVE group.\(^13\) In contrast, Wilms et al.\(^14\) found that PVE in minipigs is the more effective technique to increase the FRL. Thus, the question remains of whether PVE or PVL is superior in inducing a liver regeneration response. To elucidate this question, we used our rabbit model\(^15\) in which PVE and PVL can be performed easily to study the hypertrophy response of both procedures. The rabbit liver is very suitable for this purpose because the rabbit liver consists of 3 cranial liver lobes and 1 caudal liver lobe. The caudal lobe accounts for approximately 20% of total liver volume (TLV), corresponding to the volume of FRL that would require portal vein occlusion in humans. Furthermore, like in the clinical situation, repeated computed tomography (CT) volumetry can be performed readily in the rabbit, because CT can easily identify the caudal liver lobe. The aim of this study was to compare the hypertrophy response of the liver after PVL or PVE in this rabbit model.
Materials and methods

Animals
The experimental procedures were approved by the animal ethics and welfare committee of the Academic Medical Center, University of Amsterdam, The Netherlands. Female New Zealand white rabbits with a mean weight of 3,336 g (range, 3,130-3,830; Harlan, France) were acclimatized for ≥7 days under standard laboratory conditions. They were individually housed with a 12-hour light–dark cycle and fed standard chow ad libitum.

Study design
Twenty rabbits were divided into a PVE group (n = 10) and a PVL group (n = 10). Both groups were divided in 2 subgroups of 5 rabbits. The first subgroup was humanely killed after 7 days and the other after 14 days to collect histologic specimens.

CT volumetry
All animals underwent a multiphase CT (Brilliance 64-channel; Philips, Eindhoven, The Netherlands) before PVE and PVL. The rabbits were anesthetized with intramuscular administration of ketamine (25 mg/kg body weight, Nimatek; Eurovet, Bladel, The Netherlands) and medetomidine (0.2 mg/kg body weight, Dexdomitor; Orion, Espoo, Finland), 0.8 mL total volume, and placed in supine positionon the CT table. After acquisition of a baseline scan, 3 mL of contrast agent (Visipaque; GE Healthcare, Waukesha, WI) was injected in an ear vein followed by infusion of 4 mL of sterile physiologic saline (Baxter, Deerfield, IL). A scan was performed 15 (arterial phase), 30 (portal phase), and 45 (venous phase) seconds after infusion of contrast agent. In the first subgroup, CT was repeated on days 3 and 7 and in the latter subgroup on days 10 and 14, after which the rabbits were humanely killed. The total liver and the caudal liver lobe were delineated manually and TLV and caudal liver volume (CLV) were calculated by integrated software (Mx-View 3.52; Philips Medical Systems). CLV before PVE was expressed as percentage of TLV using the formula:

\[
\text{%CLV}_{\text{pre-PVE}} = \frac{\text{CLV}_{\text{pre-PVE}}}{\text{TLV}_{\text{pre-PVE}}} \times 100\%
\]

After PVE, %CLV was calculated using the formula:

\[
\text{%CLV}_{\text{post-PVE}} = \frac{\text{CLV}_{\text{post-PVE}}}{\text{TLV}_{\text{post-PVE}}} \times 100\%
\]

The degree of hypertrophy was calculated by subtracting the %CLV_{\text{pre-PVE}} from the %CLV_{\text{post-PVE}} on a certain time point.
Portogram
A portogram was acquired before PVE or PVL, immediately after PVE or PVL, and before animal sacrifice (on days 7 and 14).

Procedures of PVE and PVL
Rabbits were anesthetized by intramuscular injection (1.3 mL) of ketamine (25 mg/kg) and dexdomitor (0.1 mg/kg). Before operation, buprenorphine (0.03 mg/kg body weight, Temgesic; Reckitt Benckiser Healthcare Limited, Hull, UK) and enrofloxacin (0.2 mg/kg body weight, Baytril; Bayer Healthcare, Berlin, Germany) were administered subcutaneously. The eyes were protected against drying out with eye cream (Oculentum simplex; Pharmachemie B.V., Haarlem, The Netherlands). The animal was placed in supine position and 1-2% isoflurane (Forene; Abbott Laboratories, Kent, UK) mixed with O2/air (0.5:0.5 L/min) was used to maintain anesthesia. For the PVL procedure, a midline laparotomy was performed, and the main portal branch to the cranial liver lobes was ligated just above the junction of the portal branch to the caudal liver lobe using a mersilene 4.0 ligature. For the PVE procedure, a branch of the inferior mesenteric vein was cannulated with an 18-gauge catheter (Hospira Venisystems, Lake Forest, IL) after a midline laparotomy. A Renegade 3 Fr microcatheter (Boston Scientific, Natick, MA) with a Transend-ex 0.014 inch wire (Boston Scientific) was inserted subsequently into the portal vein. The catheter was introduced into the portal main branch to the cranial liver lobes, bypassing the portal branch to the caudal liver lobe. A mixture of contrast agent with 90--180 mm polyvinylalcohol particles (Cook, Bloomington, IN) was injected until flow ceased, followed by the positioning of 3 platinum coils (5 and 6 mm, Tornado Embolization Microcoil; Cook). We chose to use this embolization material, because we and others already used this material in the clinical setting. The inferior mesenteric vein was closed subsequently with a ligature. The peritoneum was closed with a running Vicryl 4.0 suture and the skin with interrupted mersilene 3.0 U-sutures. The rabbits were given the antibiotics (0.02 mg/kg Baytril; Bayer Healthcare) subcutaneously once a day for 3 days postoperatively.

Assessment of liver damage and function
Blood samples were drawn before, 3 hours after, and 3 days after portal vein occlusion, and on the day of killing. An additional sample was drawn on days 10 and 14 in the survival subgroup. Plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), bilirubin, albumin, and the prothrombintime were determined by routine laboratory assays. Before PVE or PVL and on the day of sacrifice, an indocyanine green (ICG) clearance test was performed to assess liver function. Two 22-gauge venflon catheters were placed in the ear vein and in the contralateral ear artery. Freshly prepared ICG was injected into the ear vein (12.5-mg ICG PULSION; PULSION Medical Systems, Munich, Germany; dissolved in 5 mL of sterile water). Blood samples were obtained before and 1-6 minutes after
ICG injection. Plasma samples were diluted (250 mL plasma in 600 mL of 1% bovine serum albumin in 0.9% NaCl) and measured spectrophotometrically at 805 nm (Uvikon 850; Kontron Instruments, Munich, Germany). The ICG disappearance constant (k) was derived from the slope of the semilogarithmic decay curve. Accordingly, the ICG plasma disappearance rate (PDR, %/min) was calculated using the formula:

\[ PDR = k \times 100 \]

Total liver weight and wet: dry weight ratio
After killing, the total liver and the caudal liver lobe were weighed. To demonstrate that the increase in liver volume was not due to edema, the percentage of water was determined in caudal and left lateral liver lobe biopsies. The specimens were weighed directly after sacrifice (wet weight) and stored subsequently in a stove at 60°C. After 4 weeks, the specimens were weighed again (dry weight). The percentage of water was calculated by the formula: (wet weight - dry weight x 100)/ wet weight.

Mediators of liver regeneration
The cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-6 were assessed by an enzyme-linked immunosorbent assay in homogenized liver tissue of the caudal and left lateral liver lobes using polyclonal TNF-α and IL-6 goat anti-rabbit antibodies (USCN Life, Wuhan, China) according to the manufacturer’s instructions.

(Immunohistochemistry
Paraffin sections of the caudal and left lateral liver lobes were fixed in buffered formalin, dehydrated in graded ethanol and xylene, and stained with hematoxylin and eosin (H&E). The H&E stained slides were scored for necrosis, inflammation, atrophy/sinusoidal dilatation, and edema using an ordinal scale: grade 0, none; grade 1, mild; grade 2, moderate; and grade 3, severe. All slides were scored by an experienced pathologist in a blinded fashion. Additionally, sections were immunostained with anti-Ki-67 antibodies (monoclonal mouse anti rat Ki-67 antigen, clone MIB-5; Dako Cytomation, Carpentaria, CA) and with antibodies against macrophages (monoclonal mouse anti-rabbit macrophage, clone RAM11; Dako Cytomation). The immunostained sections were counterstained with hematoxylin. Ki-67-and hematoxylin-positive cells were counted in 10 medium power fields (20 magnification) per section using Image J software (National Institutes of Health, Bethesda, MD). The proliferation index was defined as the percentage of Ki-67-positive hepatocytes per total hepatocytes in the field of view. The pixels in 10 fields of view (20 magnification) occupied by macrophages was determined by Image J software and expressed as percentage of the total amount of pixels in the field of view.

Statistical analysis
Statistical analysis was performed with Statistical Package for Social Sciences (SPSS, Chicago, IL). CT volumetry data were compared using a linear mixed model analysis.
based on ranked data. The separate time points and other continuous data were compared by the Mann-Whitney U test. Correlation between variables was tested using the Spearman’s rank correlation coefficient. All statistical tests were 2 tailed, and differences were considered significant when \( p<0.05 \). Data were tested for normal distribution and equal variances and expressed as mean values ± standard deviations unless otherwise stated.

Results

Assessment of portal vein occlusion after PVE and PVL

In all rabbits, a portogram was performed before PVE and PVL, depicting portal perfusion of the whole liver. Directly after embolization or ligation, the portogram showed total occlusion of the portal blood flow to the cranial liver lobes in all rabbits (Figure 1). Total occlusion persisted in both groups 7 days post-PVE and PVL. On day 14, however, collateral formation was visible clearly in all 5 rabbits of the PVL group, resulting in extensive parenchymal perfusion of the cranial lobes. In the PVE group, partial revascularization was found of the main portal trunk to the cranial liver lobes in 3 of the 5 rabbits, but only a little parenchymal perfusion was observed. In the other 2 rabbits, the portal vein to the cranial liver lobes was still occluded completely.

PVE- and PVL-induced hypertrophy response

As demonstrated in concordance with the findings in a previous study,15 a strong positive correlation was found between liver volume based on CT volumetry and liver weight (Spearman’s \( r =0.91; \ p<0.001 \)). The \%CLV before occlusion was 22% ± 2% in the PVE group versus 21% ± 3% in the PVL group (\( p = .19 \)). The \%CLV increased to 28% ± 4%, 33% ± 4%, 34% ± 3%, and 34% ± 6% on days 3, 7, 10, and 14 in the PVL group and to 29% ± 3%, 38% ± 3%, 41% ± 1%, and 42% ± 2% in the PVE group, respectively (\( p<0.001 \) in both groups) and was greater after PVE than after PVL (\( p=.001 \)). The mean degree of hypertrophy after 14 days was 15% ± 4% in the PVL and 20% ± 2% in the PVE group (\( p=.016 \)). When comparing the groups on the individual time points, there was already a difference in \%CLV on day 7 in favor of the PVE group which reached significance on days 10 and 14 (\( p<.02 \)).

The percentage of Ki-67-positive hepatocytes in the caudal liver lobe was greater in the PVE group (20% ± 6%) compared with the PVL group (7% ± 5%; \( p=.016 \)) on day 7. On day 14, both groups showed the same amount of proliferating hepatocytes (Figure 2). Wet:dry ratios showed no differences in water content between the PVE and PVL group on both days, indicating that the enhanced volume gain in the PVE group was not from edema.

CT volumetry data showed that PVE led to a greater hypertrophy response than PVL. This finding is supported by the greater number of proliferating hepatocytes found in the PVE group.
Before the intervention the total portal tree is clearly visible. The narrowings of the portal vein in the PVL group (white arrowheads) are spasms of the vein as a consequence of the placement of the suture material for subsequent ligation. Directly after portal vein occlusion there is no portal blood flow to the cranial liver lobes visible anymore, indicating a successful procedure. At day 14, collateral formation was clearly visible in all PVL rabbits (black arrowheads) with marked parenchymal perfusion. In the PVE group 3 of the 5 rabbits showed little recanalization in the main trunk to the cranial liver lobes, but with no or (in this rabbit) minimal parenchymal perfusion.
Figure 2. Ki-67 staining. At day 7, there were significantly more proliferating hepatocytes in the caudal liver lobe in the PVE group compared with the PVL group (*p = .016).

**Determination of liver damage and function**

In both groups, parameters of liver damage in plasma showed a transient increase with a peak after 3 hours (LDH) or 3 days (AST, ALT), which returned to baseline values on day 7 (Figure 3). The H&E slides showed normal liver architecture in the caudal liver lobe after both procedures. In the PVE groups, the PVA particles were visible clearly within the portal veins of the atrophic liver lobe. A foreign body reaction characterized by multinucleated giant cells was present around these particles. No necrosis or edema was observed in either group. Diffuse infiltration of inflammatory cells (lymphocytes and granulocytes) and sinusoidal dilatation/atrophic trabeculae were observed in the atrophic lobes in the PVE and PVL group to a similar degree (Figure 4). Plasma bilirubin and albumin concentrations, prothrombin time, and ICG-PDR were measured to assess the synthetic and clearance functions of the liver. The bilirubin levels and prothrombin time were stable over time. Plasma albumin concentration and ICG-PDR showed a small decrease in the first week in both groups, which was restored in the second week (Figure 5).

**Mediators of liver regeneration**

Kupffer cells produce cytokines, which are important for liver regeneration. There were more Kupffer cells in the atrophic liver lobes of the PVE group compared with the PVL group on day 7 (p = .032; Figure 6). In contrast with the PVL group, there were more Kupffer cells present in the atrophic, embolized liver lobe compared with the hypertrophic, nonembolized liver lobe in the PVE group (p < .02 on days 7 and 14). There were no differences in IL-6 and TNF-a level between the PVE and PVL groups on days 7 and 14, respectively.
Figure 3. Liver damage. Plasma levels of LDH (A) and ALT (B) showed an increase after 3 days and 3 hours, respectively, in both groups, after which they returned to baseline values.

Figure 4. Histology. H&E slides of the atrophic liver lobe 7 days after portal vein embolization (PVE) and portal vein ligation (PVL) show sinusoidal dilatation after both procedures. The embolization material with foreign body reaction is clearly visible in the portal vein (arrow).
Figure 5. Liver function. Plasma albumin levels (A) and the ICG-PDR (B) showed a transient, mild decrease after both procedures and returned to baseline levels within 14 days.

Figure 6. Macrophages/Kupffer cells. At day 7 the RAM11-positive area was significantly larger in the atrophic liver lobe after PVE compared with PVL (*P = .032). In the PVE group, the area was larger in the atrophic compared with the hypertrophic liver lobe on days 7 and 14 (#P = .08 and .016, respectively).
Discussion

In this study, we compared the effects of ligation versus embolization of the portal vein on the hypertrophy response of the FRL in a rabbit model. The most effective occlusion technique is unknown currently. Every animal model has its strengths and weaknesses dictated by factors as species-dependent morphology and practical issues. The anatomy of the rabbit liver is unique in that the cranial and caudal lobes are separate, and the caudal lobe accounts for 20% of the TLV. These configurations make the rabbit liver very suitable to examine selective portal vein occlusion and the resulting regenerative response. In addition, a great advantage of our model is that each rabbit serves as its own control, because of the repeated CT volumetry measurements. This ability to repeat CT volumetry is comparable with the clinical situation and the results are statistically stronger. We showed that PVE induces a significantly greater hypertrophy response than PVL as assessed by CT volumetry and supported by liver weight measurements and the amount of proliferating hepatocytes. These results are comparable with the results of a study in minipigs of Wilms et al,\textsuperscript{14} in which the hypertrophy response was assessed by liver to body weight.

In our study, the amount of proliferating hepatocytes was significantly greater in the PVE group on day 7. In contrast to this result, Furrer et al\textsuperscript{13} showed in a rat model that the number of proliferating hepatocytes in the regenerating liver lobe was significantly less in the PVE group compared with the PVL and PH groups 48 hours after the intervention; this difference disappeared after 72 hours. In our study, the amount of proliferating hepatocytes was significantly greater in the PVE group on day 7. Although we performed the measurement on different time points, it is not likely to assume that more proliferating hepatocytes would occur in the PVE group at a later time point in this rat study.

Several factors may be considered to explain the difference in hypertrophy response after PVE and PVL. First, formation of collateral portal vessels leading to parenchymal portal reperfusion after PVL seems a likely explanation. This phenomenon was described previously by Denys et al\textsuperscript{19} and was confirmed by the abovementioned study in minipigs in which duplex ultrasonographic measurements showed that the portal branches were occluded for a greater time duration after PVE and that collateral formation seemed to be the cause of less effective regeneration in the PVL group. In our study, portal venous collateral formation was visible clearly 14 days after PVL with concomitant reperfusion of the parenchyma of the previously ligated liver lobes. In the PVE group, no collateral formation was seen, but minor recanalization of the main trunk of the portal vein to the cranial lobes was visible, which, however, did not lead to substantial parenchymal reperfusion of the embolized liver lobes.

Interestingly, the hypertrophy response after PVE was already greater before collateral formation was visible in the PVL group. Therefore, there must be another mechanism for the hypertrophy response and the difference after PVE or PVL. This may be sought...
in the cytokine response. Kupffer cells produce IL-6 and TNF-a, which are important mediators of liver regeneration.\textsuperscript{13} One of the hypotheses of Furrer et al\textsuperscript{13} to account for the superiority of PVL over PVE was the observed entrapment of macrophages in the embolized liver lobes owing to a foreign body reaction caused by the embolization material. This process would lead to a decreased accumulation of macrophages and thus decreased production of cytokines in the regenerating liver lobe. In our study, we used a different embolization material. Nevertheless, we also found significantly more Kupffer cells in the atrophic, embolized liver lobes compared with PVL; however, in contrast to the study by Furrer et al,\textsuperscript{13} the regenerative response after PVE was greater than after PVL. We did not find a difference in IL-6 and TNF-a levels in the regenerating lobes of the PVE and PVL groups after 7 and 14 days postocclusion. It could be that we missed the peak of the cytokine response because this peak probably occurs earlier in rabbits.

Liver damage in our study was mild after PVE as well as after PVL as shown by plasma AST, ALT, and LDH levels and the absence of parenchymal necrosis. Liver function as assessed by plasma bilirubin and albumin levels, prothrombin time, and ICG clearance rate was not markedly affected. These findings are in accordance with other reports\textsuperscript{14,21} and confirm that both techniques can be performed safely, without a risk of liver failure.

Regarding the rabbit model used in our study, several remarks are in order. First, the rabbit liver consists of 4 main liver lobes connected by parenchymal bridges. The parenchymal contact between the occluded and nonoccluded lobes is, therefore, less extensive than in the human liver. The formation of a collateral portal venous flow is more likely to occur in the human liver because of the close contact of the right and left liver segments, which suggests that the hypertrophy response after PVL in humans would even be less. A limitation of this model is the different approach for PVE. In humans, the portal vein is usually catheterized via a percutaneous transhepatic approach, whereas in our rabbit model a laparotomy was used to access the portal vein. For reasons of comparing only the effects of PVL and PVE, it is preferable to perform a laparotomy in both instances in this model.

In conclusion, based on the results in our rabbit model, the regenerative response after PVE seems to be superior to PVL, at least in this rabbit model. Our findings may have important implications in man.
References