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A rabbit model for selective portal vein embolization

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Abstract

Background: Portal vein embolization (PVE) is a technique to increase future remnant liver volume. A standardized animal model, resembling the clinical PVE procedure, is needed to clarify some of the unresolved issues surrounding PVE. For this purpose we developed a new rabbit model for PVE.

Materials and methods: Twenty female New Zealand white rabbits were allocated to two protocols, each containing two subgroups. Eighty percent of the liver portal venous system was embolized with polyvinylalcohol particles and coils (protocol 1: 300–500 mm particles and one coil; protocol 2: 90–180 mm combined 300–500 mm particles and three coils). In all rabbits CT-volumetry, ICG clearance test, blood sampling, and portography were performed prior to PVE and at d 7 and 14. Additional blood sampling and CT volumetry was done on d 3 and 7.

Results: PVE was technically feasible in the rabbit. CT-volumetry demonstrated a strong correlation with actual liver weight and volume measured at sacrifice. The hypertrophy response was highest at d 7 in both protocols, which was consistent with the amount of proliferating hepatocytes. Protocol 2 showed less revascularization of the portal venous system and demonstrated the highest hypertrophy response. Comparable to the clinical situation, only a small, transient increase in transaminases was observed. There were no changes in liver function parameters after PVE. Histopathologic findings in the rabbit livers were comparable to those found in human livers.

Conclusion: We successfully devised a rabbit model for PVE, which resembles the human clinical situation.
Introduction

In 1920, Rous and Larimore described their unforeseen observation that ligation of portal branches in a rabbit resulted in atrophy of the ligated liver lobes and a compensatory hypertrophy of the remaining organ.\(^1\) Although they recognized that this observation could explain the changes in liver morphology seen in liver lesions that cause disturbance of local portal flow, they did not foresee that this observation would lay the foundation for preoperative portal vein embolization (PVE). PVE was clinically introduced in 1986 to increase the number of patients eligible for liver resection.\(^2\) It induces atrophy of the embolized, tumor bearing liver segments while compensatory hypertrophy occurs in the nonembolized lobes, thereby increasing future remnant liver volume and function before major liver resection is undertaken. Although the beneficial effect of preoperative PVE for the induction of contralateral hypertrophy has been demonstrated by many studies,\(^3\)\(^-\)\(^7\) several issues remain uncertain or controversial,\(^8\) including the mechanisms underlying the atrophy-hypertrophy complex, the best occlusion technique (PVE or portal vein ligation (PVL)),\(^8\) the best embolization material,\(^8\) the effect of underlying liver disease on the hypertrophy response,\(^6\)\(^,\)\(^9\) and the effect of PVE on tumor progression.\(^10\)

The available information regarding the mechanisms behind the atrophy-hypertrophy complex is mostly derived from PVL models in the rat.\(^11\) Since a few years, there is growing evidence that the mechanisms behind liver regeneration after PVE might differ from those after PVL.\(^12\)\(^-\)\(^14\) A standardized animal model, resembling the clinical PVE procedure, is necessary to clarify some of the unresolved issues surrounding PVE and to subsequently improve the technique of PVE.

Recently, two new animal models for PVE were published. Furrer et al. described a rat model using embospheres as embolization material.\(^13\) Although an elegant model, the size of the rat has its limitations. Not all embolization materials can probably be used in this model, especially the use of coils is difficult. The procedure requires introduction of a relatively large needle in the central portal vein. In addition, the portal vein to the nonembolized segments was clamped during the embolization procedure. All these factors might influence the hemodynamics in the nonembolized liver lobes. Furthermore, noninvasive volumetric assessment of the different liver lobes is difficult in this model. Wilms et al. described a PVE model in a mini-pig,\(^12\) which is comparable with the human situation. Disadvantages are the costs and labour-intensiveness involved in experiments with mini-pigs.

The aim of this study was therefore to devise a new model for PVE in the rabbit, which resembles the clinical procedure.
Materials and methods

Animals
Experimental protocols were approved by the Institutional Animal Ethics Committee. Female New Zealand white rabbits (Harlan, Horst, The Netherlands) with a mean weight of 3,265 g were acclimatized for 2 weeks under standardized laboratory conditions in a temperature-controlled room with a 12 h-light/dark cycle and with access to standard chow and water ad libitum.

Figure 1. Anatomy of the rabbit liver (A). The rabbit liver is subdivided into four main lobes: these are the caudal liver (CL) lobe and three cranial liver lobes, comprising the left lateral (LL), left medial (LM), and right liver (RL) lobes. A portogram of the rabbit liver is depicted in (B). The portal vein branch to the caudate lobe takes off to the right. More cranially, the portal vein bifurcates into the right and left portal vein branches, after which the left portal vein divides into the medial and lateral segmental branches.

Experimental design
The rabbit liver is subdivided into four main lobes: a caudal liver lobe and three cranial liver lobes (Figure 1). We embolized 80% of the liver by embolizing the portal system of all cranial liver lobes. For this experiment, the combination of PVA particles and coils was used, since this is also used in the clinical setting in our institution. Rabbits (n=20) were allocated into two protocols. In protocol 1, PVE was performed using one PVA particle size combined with one coil. In protocol 2, the liver was embolized using two different sizes of PVA particles followed by three coils. Both protocols included two subgroups (n=5 per subgroup) in order to obtain liver tissue on different time points. In the first subgroup, CT volumetry was performed before PVE and on d 3 and 7 after PVE. Rabbits were sacrificed on d 7. In the second subgroup, CT volumetry was performed before PVE and on d 10 and 14 after PVE, followed by sacrifice on d 14. Blood samples were drawn before PVE, as well as 3 h and 3 d after PVE. Additional blood samples were taken at the time of CT volumetry. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed as well established liver damage parameters by routine clinical chemistry. Prothrombin time (PT) and albumin (Alb) were used as indirect parameters of liver synthetic function, whereas plasma bilirubin
(bili) was used as an indirect measure of hepatic uptake and excretory function. In addition the indocyanine green (ICG) clearance test was performed as a quantitative dynamic liver function test prior to PVE and on the day of sacrifice. After sacrifice, liver weight was measured and liver biopsies of both embolized and the nonembolized liver lobe(s) were taken.

**Portal vein embolization**

Animals were anesthetized by intramuscular injection of 25.0 mg/kg ketamine (Nimatek; Eurovet, Bladel, The Netherlands) and 0.2 mg/kg dexmedetomidine (Dexdomitor; Orion Corp., Espoo, Finland). After subcutaneous injection of 0.03 mg/kg buprenorphine (Temgesic; Reckitt Benckiser Healthcare Ltd., Hull, UK) and 0.2 mg/kg Baytril (Bayer Healthcare, Berlin, Germany), the rabbit was placed in a supine position. Isoflurane 1%–2% (Forene; Abbott Laboratories, Kent, UK) with O2/air (0.5:0.5 L/min) was used to maintain anaesthesia. Heart rate and arterial oxygen saturation were measured by pulse oximetry (Hewlett Packard M1165A model 56S; Andover, MA) throughout the procedure. The embolization was performed by an interventional radiologist with over 10 years experience. After midline laparotomy, a branch of the inferior mesenteric vein was cannulated with an 18 gauge catheter (Hospira Venisystems, Lake Forest, IL). A Renegade 3 Fr microcatheter (Boston Scientific, Place Natick, MA) with a Transend-ex 0.014 inch wire (Boston Scientific, Natick, MA) was subsequently introduced into the portal vein. A portogram was made to identify the individual portal branches (Figure 1). The microcatheter was positioned in the main portal branch supplying the cranial liver lobes after passing the portal branch to the caudal liver lobe. In protocol 1, a mixture of contrast (Visipaque; GE Healthcare, Waukesha, WI) with 300–500 mm PVA particles (Cook, Bloomington, IN) was injected until flow ceased, followed by the positioning of a platinum coil (6 mm, Tornado Embolization Microcoil; Cook, Bloomington, IN). In the second protocol, 90–180 mm PVA particles were used followed by 300–500 mm particles and three platinum coils (5 and 6 mm). Portography was repeated to confirm that the cranial liver lobes were deprived of portal blood flow. The inferior mesenteric vein was subsequently closed with a ligature. Rabbits were given Baytril 0.02 mg/kg subcutaneously once a day for 3 d postoperatively.

**CT volumetry**

After anesthesia, a multiphasic CT scan was performed using a 64-slice CT scan (Brilliance 64-channel; Philips, Eindhoven, The Netherlands). Rabbits were placed in supine position. After blank series, a contrast enhanced scan was performed 15 s (arterial phase), 30 s (portal phase), and 45 s (venous phase) after contrast injection (4 mL Visipaque; GE Healthcare, Waukesha, WI), followed by 3 mL NaCl. 3D-reconstructions of the liver were made using reconstructed 2 mm axial slices (Figure 2). The total liver and the caudal liver lobe were manually delineated and total liver volume (TLV) and caudal liver volume (CLV) were calculated. CLV before PVE was expressed as percentage of TLV using the formula:
After PVE, %CLV was calculated using the formula:

\[
%CLV_{\text{post-PVE}} = \frac{CLV_{\text{post-PVE}}}{CLV_{\text{pre-PVE}}} \times 100\%
\]

After PVE, increase in CLV was calculated using the formula:

\[
\text{increase CLV} = \left( \frac{CLV_{\text{post-PVE}} - CLV_{\text{pre-PVE}}}{CLV_{\text{pre-PVE}}} \right) \times 100\%
\]

**Indocyanine green clearance test**

A 22 gauge Venflon was 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, München, Germany) in 5 mL sterile water. Blood samples were obtained before and 1, 2, 3, 4, 5, and 6 min 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, München, Germany). The ICG disappearance constant (k) was derived from the slope of the semilogarithmic decay curve. Accordingly, the ICG plasma disappearance rate (ICG-PDR, %/min) was calculated using the formula: PDR = k x 100⁻¹.

**Determination of liver weight and liver volume**

After sacrifice, the liver was weighed using a precision scale (Sartorius, Goettingen, Germany). Liver volume was measured using a measuring jug filled with water by subtracting the volume of the water alone from the volume of the water including the liver.

**Liver regeneration**

Immunostaining for Ki-67 was performed to identify proliferating hepatocytes. Sections were deparaffinized and endogenous peroxidase activity was blocked by incubation in 0.3% H2O2 in methanol. Subsequently, sections were boiled in 10 mM Tris/1 mM EDTA buffer (pH=9.0) for 20 min, and incubated for 1 h at room temperature with monoclonal mouse anti-rat Ki-67 antibody (MIB5; DAKO Cytomation, Glostrup, Denmark) with known cross reactivity for rabbit tissue. After incubation with anti-mouse poly-horseradish peroxidase (Immunologic, Duiven, The Netherlands) for 30 min, diaminobenzidine (DAB, Sigma-Aldrich, St. Louis, MO) was used to label peroxidase complexes. Sections were counterstained with hematoxylin. Immunolabeled and hematoxylin-positive nuclei were quantified with ImageJ software (NIH, Bethesda, MD). The mitotic index was defined as the percentage of Ki-67 positive hepatocytes averaged over 5 low power fields (10 magnification) per slide.
Histopathologic evaluation

Histologic examination of the atrophic lobes of the rabbit liver demonstrated similar features as in biopsies taken from human atrophic liver segments after PVE (Figure 8A and B). PVA particles were clearly visible within the portal veins. A foreign body reaction characterized by multinucleated giant cells was found around the PVA particles. The liver parenchyma demonstrated sinusoidal dilatation with atrophic hepatocyte trabeculae. The areas of trabecular atrophy were unevenly distributed throughout the liver parenchyma. Diffuse infiltration of inflammatory cells was seen within the liver parenchyma, both periportal and centrilobular (although less pronounced in rabbit tissue than in human tissue). The hypertrophic liver segments were characterized by intact liver architecture in both rabbits and humans. There was little sinusoidal dilatation but not as pronounced as in the atrophic liver lobe. There was some influx of inflammatory cells in the periportal region, which was more prominent in the human liver tissue (Figure 8C). Overall, the histopathologic features of both the atrophic and hypertrophic liver parenchyma of the rabbit closely resembled the histology of the human liver after PVE.

Statistical analysis

Statistical analysis was performed with GraphPad Prism (Graph-Pad Software, San Diego, CA) and Statistical Package for Social Sciences (SPSS 16.02, SPSS Inc., Chicago, IL). CT volumetry data were compared using a mixed model analysis based on ranked data. 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 two-tailed and differences were considered significant at a p value <0.05. Data were expressed as means ± SD.

Results

PVE procedure

One rabbit was excluded in protocol 1 because the coil migrated into the portal branch of the caudal lobe during the procedure. In all remaining rabbits, portography directly after embolization confirmed complete portal vein occlusion of the cranial liver lobes and normal flow in the caudal lobe (Figure 3A). In three out of the five rabbits in protocol 1, the portogram at d 7 revealed restoration of flow in the main portal branches to the cranial liver lobes distal of the coil and additional perfusion of the liver parenchyma, suggesting revascularization of the occluded portal venous system (Figure 3B). At d 14, three out of the four rabbits showed considerable parenchymal perfusion of the cranial liver lobes. In protocol 2, the portogram at d 7 showed minimal portal flow to the cranial liver lobes without parenchymal perfusion in two of five rabbits (Figure 3C). After 14 d,
also 2 of the five rabbits showed minimal revascularization at the site of the coils without parenchymal perfusion. However, one other rabbit demonstrated minimal parenchymal perfusion of the cranial lobes (Figure 3D). No formation of collateral vessels to the cranial liver lobes was observed.

**Figure 2.** Example of CT volumetry in the rabbit. (A) and (B) show a contrast enhanced CT scan. Because of the anatomical position, the caudal liver lobe (green) can be clearly distinguished from the other liver lobes. (C) depicts a three-dimensional CT scan reconstruction of the rabbit liver depicting total liver (yellow) and the caudal liver (green).

**Validation of CT volumetry in the rabbit**

The total liver volume measured by CT volumetry correlated well with actual liver volume and liver weight measured after sacrifice (Spearman’s r =0.77 and 0.84, respectively). In addition, caudal liver volume measured by CT volumetry showed a strong correlation with actual caudal liver volume and weight (Spearman’s r=0.85 and 0.93, respectively), indicating that CT volumetry is an accurate method for noninvasive measurement of liver volume in the rabbit (Figure 4). Liver volume measured by CT volumetry was, however, significantly larger than liver weight and liver volume measured after sacrifice.

**Hypertrophy response after PVE**

Before PVE, CLV expressed as percentage of total liver volume (%CLV) was 21.2% ± 2.0% and 21.8% ± 2.0%, for protocols 1 and 2, respectively (Figure 5A). When we
compared the %CLV between both groups, over time a significant difference was found between the two protocols showing that protocol 2 is more effective in increasing % CLV ($p=0.013$). When the separate time points were analyzed, a significant difference was found on d 10 and 14 ($p=0.007$ and $p=0.002$, respectively) in favour of protocol 2 (Figure 5B). There was a significant increase of CLV in both protocols after 3 and 7 d. Only little additional increase was observed after 10 and 14 d.

**Figure 3.** Examples of portograms made after PVE. A successful procedure is demonstrated in (A), showing only portal flow to the caudal lobe; (B) demonstrates a portogram of a rabbit in protocol 1, 7 d after PVE. Revascularization is clearly visible; (C) shows an example of minimal flow distal of the coil with no parenchymal perfusion 7 d after PVE (protocol 2); (D) shows a portogram made 14 d after PVE in protocol 2 showing minimal parenchymal perfusion.

### Hepatocellular damage after PVE

In protocol 2, a significant but mild elevation in AST level was found 3 h and 3 d after PVE compared with pre-PVE baseline values ($p=0.008$ and $p=0.011$, respectively). In protocol 1, a mild elevation was seen only after 3 d ($p=0.008$). Plasma ALT levels were significantly elevated in protocol 1 at 3 d ($p=0.012$) and in protocol 2 at 3 h, 3 d, and 7 d after PVE ($p=0.008$, $p=0.008$, and $p=0.043$, respectively). Plasma AST/ALT levels during the experiments are shown in Figure 6.

### Liver function after PVE

No substantial changes in biochemical liver function tests as well as in the ICG-PDR were observed after PVE in both protocols ($p > 0.05$).
Liver regeneration

In both protocols a significant increase in proliferating cells within the nonembolized liver lobe was observed compared with the embolized liver lobes (Figure 7). At d 7, the percentage of proliferating cells in the hypertrophic lobe was significantly higher in protocol 2 compared with protocol 1 (p=0.032). At d 14, no significant difference was found.

![Figure 4. Validation of CT-volumetry in the rabbit. There was a strong and significant correlation between total liver volume (TLV) measured by CT volumetry and actual liver volume (A) and liver weight (B). In addition, caudal liver volume (CLV) measured by CT volumetry showed a strong correlation with actual caudal liver volume (C) and weight (D) measured after sacrifice.](image)

Histopathologic Evaluation

Histologic examination of the atrophic lobes of the rabbit liver demonstrated similar features as in biopsies taken from human atrophic liver segments after PVE (Figure 8A and B). PVA particles were clearly visible within the portal veins. A foreign body reaction characterized by multinucleated giant cells was found around the PVA particles. The liver parenchyma demonstrated sinusoidal dilatation with atrophic hepatocyte trabeculae. The areas of trabecular atrophy were unevenly distributed throughout the liver parenchyma. Diffuse infiltration of inflammatory cells was seen within the liver parenchyma, both periportal and centrilobular (although less
pronounced in rabbit tissue than in human tissue). The hypertrophic liver segments were characterized by intact liver architecture in both rabbits and region, which was more prominent in the human liver humans. There was little sinusoidal dilatation but not tissue (Figure 8C). Overall, the histopathologic features as pronounced as in the atrophic liver lobe. There was of both the atrophic and hypertrophic liver parenchyma of the rabbit closely resembled the histology of the human liver after PVE.

Figure 5. Increase in %CLV (A) and CLV (B) measured by CT volumetry. There was a significant difference in % CLV between the two protocols over time, showing that protocol 2 is more effective in increasing the % CLV (p=0.013) (A). When the separate time points were analyzed, a significant difference in % CLV was found on d 10 and 14 (A). In both protocols, the increase in CLV was most pronounced in the first week after PVE, with only little additional increase on d 10 and 14 (B).

Figure 6. Transient elevation of plasma ALT/AST levels after PVE. Statistically significant differences compared with pre-PVE baseline values are indicated by an asterisk.

Discussion
In this study we describe a rabbit model for PVE, which resembles the clinical situation. After initial pilot studies in which we developed the PVE procedure, pro-
Protocol 1 was performed using 300–500 mm PVA particles combined with one coil in the main branch to the cranial liver lobes. Application of PVA particles in combination with a coil is considered permanent. Protocol 1 was not successful in all animals as partial revascularization of the portal system to the cranial lobes was already observed after 7 d, resulting in less regeneration and less hypertrophy response. The coil was unable to prevent flow into the main portal branches and partial reperfusion of the liver parenchyma indicated that not all peripheral portal branches were occluded by the PVA particles. Apparently, particle size and the number of coils are crucial for the efficacy of the PVE procedure. Therefore, in protocol 2, both 90–180 and 300–500 mm PVA particles in combination with three coils were used, showing no parenchymal perfusion of the cranial lobes after 1 wk. After 14 d however, three rabbits demonstrated flow in the main cranial portal branches, which resulted in only minimal perfusion of the liver parenchyma in one rabbit. Therefore, protocol 2 (using the combination of two PVA particle sizes, with three coils) is recommended for future research. The mechanism by which revascularization in the rabbit liver occurs may be due to lysis of the clot at the site of the coils. No evident collateral vessels were identified. Since portography in patients is usually not performed during follow-up after PVE, it is uncertain whether partial revascularization also occurs in the liver.
human situation. Revascularization has been described after embolization of cerebral arteriovenous malformations using PVA particles as embolic material.\textsuperscript{17} Similar to the human situation, CT volumetry was useful in our rabbit model to assess liver growth noninvasively, and allowed us to perform repeated measurements within one rabbit, which obviously is a great advantage. Because of the anatomical position, the caudal liver lobe can be clearly distinguished from the other liver lobes. Liver volume measured by CT volumetry correlated strongly with actual liver weight and volume at time of sacrifice. Total liver volume measured by CT volumetry was, however, significantly larger than the liver weight at sacrifice. This is also observed in humans\textsuperscript{18} and can (partially) be explained by the fact that blood volume is not included after sacrifice, while it is included in vivo and measured by the CT scan. Comparable with the clinical situation, only a small, transient increase in transaminases was observed post-PVE. Findings at histologic examination of the atrophic and hypertrophic liver lobes resembled those in humans and rabbits. As expected, Ki-67 staining showed a significant increase of proliferating cells in the caudal liver lobe, which was more pronounced at d 7 after PVE. This is in agreement with the findings of CT volumetry, which showed less volume increase in the second week. Many different embolization materials are clinically used, including ethanol, fibrin glue, gel foam, gelatine sponge particles, polyvinyl alcohol particles (PVA), embosphere particles, and various coils. Different responses regarding inflammation and the degree of hypertrophy have been observed with different embolization materials.\textsuperscript{19,20} Comparison between the different embolization materials is difficult in the clinical setting. A recent study by Lainas et al. demonstrated that although recanalization occurred, absorbable gel foam was able to induce a significant hypertrophy response.\textsuperscript{21} The results from protocol 1 confirm that although less than protocol 2, significant hypertrophy does occur within the first week despite evidence of revascularization. As underscored by Lesurtel and Belghiti, the use of absorbable embolic agents has two advantages: First, the overflow of the embolization agent in the nonembolized lobe will not impair liver regeneration in these segments. Second, complete revascularization after embolization will reduce injury of the embolized liver lobes when they are eventually not resected.\textsuperscript{22} On the other hand, the risk of tumor progression after PVE makes it important to minimize the time required to attain sufficient hypertrophy of the nonembolized liver segments to allow safe resection.\textsuperscript{10} The most effective embolization technique is currently unknown and needs further investigation. Our standardized rabbit model can be used to compare different embolization materials and clarify this issue. Controversy exists regarding the effect of PVE on tumor progression in both the embolized and nonembolized tumor lobes. A liver metastasis tumor model in the rabbit is well described using a VX2 cell line.\textsuperscript{23} This model can be combined with our PVE model to study the effect of embolization on tumor growth. In addition, liver steatosis and fibrosis can be introduced to study the effect of different parenchymal liver disease on the hypertrophy response.
Figure 8. Comparison of the histologic changes in the rabbit and human liver after PVE (H and E staining). Histologic examination of the atrophic liver lobe clearly demonstrates the PVA particles within the portal veins. A foreign body reaction (►) characterized by multinucleated giant cells is found around the PVA particles (A). The atrophic liver parenchyma demonstrates sinusoidal dilatation with atrophic hepatocyte trabeculae (B). The hypertrophic liver segments are characterized by intact liver architecture in both rabbits and humans. There is some influx of inflammatory cells in the periportal regions, which is more prominent in the human liver tissue (C).
Conclusion

The combination of two PVA particle sizes and three coils (protocol 2), creates a PVE model in the rabbit that resembles the human situation, taking into account features as histologic changes in the liver parenchyma and the hypertrophy response assessed by CT volumetry. This rabbit model provides an opportunity to perform investigations in a standardized animal model in order to improve the techniques currently used in PVE.
References