Radiological aspects of portal vein embolization

van Lienden, K.P.

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Chapter

Enhanced tumor growth after portal vein embolization in a rabbit VX2 tumor model

L.T. Hoekstra
K.P. van Lienden
J. Verheij
C.M. van der Loos
T.M. van Gulik

Submitted CVIR
Abstract

Objectives: To assess tumor growth and liver regeneration after portal vein embolization (PVE) in a rabbit hepatic tumor model.

Background data: Preoperative PVE is employed to increase future remnant liver volume through induction of hepatocellular regeneration. This event, however, potentially enhances tumor growth.

Methods: Two weeks after subcapsular implantation of a VX2 carcinoma in the cranial liver lobe, New Zealand White rabbits were allocated to a control group or PVE group (n=5/group). In the PVE group, the portal vein branch to the cranial liver lobes (80%) was embolized using particles and coils (PVE-group), leaving the caudal liver lobe (20%) free. In the tumor control group, the liver was only mobilized. CT volumetry was performed on days 3, 7, 10, and 14. Tumor growth rate (TGR), hepatocellular proliferation rate, and liver damage parameters were assessed before PVE, and on day 1, 3, 7, 10, and 14.

Results: Portography confirmed complete occlusion of the portal vein branch to the cranial liver lobes in all PVE-rabbits. The hypertrophy response and proliferation rate in the non-embolized liver lobes were significantly higher in the PVE group, which was confirmed by liver to body-weight index assessment. TGR was increased in both groups, with a significantly larger increase in the PVE-group over time (day 14: mean 34.4±4.3mL/day vs control: 24.1±7.2mL/day).

Conclusions: Tumor growth (TGR) was significantly increased after PVE in the rabbit tumor model. This finding supports the notion that PVE potentially enhances tumor growth, along with regeneration of the non-embolized liver lobe.
Introduction

Liver resection is the most effective treatment for primary or metastatic liver tumors. In advanced tumors, the extent of liver resection is restricted by the minimum volume of liver remnant required to provide sufficient postoperative liver function. Insufficient future remnant liver (FRL) is a reason why patients are considered unresectable. Preoperative portal vein embolization (PVE) is an option to increase FRL volume through induction of regeneration of the hepatocellular mass of the FRL. Following occlusion of the right or left main branch of the portal vein, atrophy of the embolized liver segments occurs while hypertrophy of the contralateral, non-embolized liver lobe is induced. PVE has shown to reduce the risk of liver failure after resection and consequently increases the number of patients who are eligible for liver resection. Although increasingly used, the mechanisms of PVE largely remain unknown. Resection is usually performed 3-6 weeks after PVE, but the exact time optimum remains controversial. There is increasing evidence that PVE not only stimulates growth of the FRL but also increases tumor size because of growth factors and cytokines released in the process of liver regeneration. Furthermore, after unilateral reduction of portal blood flow following PVE, there is a compensatory increase in blood perfusion through the hepatic artery (hepatic arterial buffer response). As liver tumors are mainly fed by arterial blood supply, these mechanisms altogether potentiate local tumor growth after unilateral embolization of the portal vein.

The challenge for future use of PVE is to limit the growth of tumor while inducing a maximum hypertrophy response in the non-embolized liver lobe. Therefore, we devised an animal model in rabbits, in which the rate of tumor growth can be assessed in relation with PVE, resembling the clinical situation. In this rabbit model, PVE is performed using the same methods and imaging protocol used in patients undergoing PVE. The combination of a VX2 liver tumor in this rabbit model allows us in addition, to explore the effects of PVE on tumor kinetics. The aim of our study is, therefore, to determine the extent and kinetics of induced tumor growth after PVE in a rabbit VX2 liver tumor model, along with assessment of the hypertrophy response of the non-embolized liver lobe.

Materials and methods

Animals

The study protocol was approved by the Institutional Animal Ethics Committee of the Academic Medical Center of the University of Amsterdam. Ten female New Zealand White rabbits (Harlan, Charles River, France) with a mean weight of 2987±149g were acclimatized for 2 weeks under standardized laboratory conditions. The VX2 cell line was obtained from Utrecht Medical Center (Utrecht, The Netherlands) for tumor implantation. VX2 tumor cell suspension was injected into the thigh muscles of the hind limb of a donor rabbit. Three weeks later, the solid tumor was harvested from the donor rabbit. In the PVE-tumor-
group, four tumor fragments of 0.5x0.5 mm were injected superficially in the subcapsular area of the left medial liver lobe using a 16-gauge angiocatheter. After removal of the angiocatheter, the liver capsule was manually compressed, followed by closure of the abdomen in two layers. Two weeks after implantation of the VX2 carcinoma, the tumor had acquired sufficient mass to be used for the experiments. Anaesthesia for tumor implantation or for PVE/laparotomy procedures, was induced by intramuscular injection of a mixture of nimetake (25.0 mg/kg body weight, Eurovet, Bladel, the Netherlands) and medetomidine (0.2 mg/kg body weight, Orion, Espoo, Finland).

Experimental design
The rabbit liver is subdivided into four main lobes: these are the caudal liver lobe and three cranial liver lobes, comprising the left lateral, left medial, and right liver lobes, each supplied by branches of the portal venous system (Figure 1). The cranial liver lobes accounting for 80% of total liver volume, are isolated from the caudal liver lobe (20%), making the rabbit liver suitable for selective occlusion of the portal vein to the cranial liver lobes, thereby inducing a compensatory hyperplasia in the caudal lobe.

Ten New Zealand White rabbits with tumor bearing livers, were divided into two groups: a control tumor group without PVE (control, n=5), and a group undergoing embolization of the portal vein to the cranial liver lobes (PVE, n=5). PVE was performed on day 0 with PVA particles 90-180 μm combined with 300-500 μm particles and 3 or 4 platinum coils, since this has shown to result in the greatest increase in volume of the non-embolized, caudal liver lobe.9,10 Furthermore, the same embolization materials are also successfully used in clinical PVE. Details about the method of PVE in rabbits have been described elsewhere.9,10

Portography was performed with a mobile C-arm Exposcop 8000 (Ziehm Imaging, Nürnberg, Germany) at laparotomy before PVE, directly after PVE and on day 14 to confirm complete occlusion of the embolized portal vein. In the control group, the liver and an access branch to the inferior mesenteric vein were mobilized without embolization, after midline laparotomy. The abdomen was closed in two layers, similar to the PVE-group.

All animals received Buprenorphine (0.03 mg/kg body weight, Reckitt Benckiser Healthcare, Hull, Great Britain) subcutaneously before surgery. Enrofloxacin (0.02 mg/kg body weight, Baytril, Bayer Healthcare, Berlin, Germany) was administered subcutaneously before operation and postoperatively from day 1 till day 3.

CT volumetry
A CT-scan was made on the day of tumor implantation, which is 14 days before embolization or laparotomy alone. Tumor growth and hypertrophy of the caudal, non-embolized liver lobe were determined in all groups by CT-volumetry following PVE or laparotomy, and on day 3, 7, 10 and 14, after which the rabbits were sacrificed. Anaesthesia was maintained with medetomidine 0.2 mg/kg and nimetake 25.0 mg/kg. The rabbits were resting in a supine position, followed by an injection of contrast solution (3 mL Visipaque, GE Healthcare, Waukesha, WI) in the ear vein, whereafter 4 mL sterile physiological saline was flushed. A
contrast-enhanced multiphasic CT-scan was performed using a 64-slice CT scan (Brilliance 64-channel, Philips, Eindhoven, The Netherlands) for the arterial phase (15s), portal phase (30s), and venous phase (45s). On each section of the CT scan, the total liver, the caudal liver lobe and the tumor(s) were delineated manually. Volumes of the total liver (TLV), the caudal liver lobe (CLV) and the tumors (TV) were calculated.

Tumor growth rate (TGR) after PVE or laparotomy was calculated by the formula: \( \frac{TV_{dx}}{TV_{d0}} \), in which \( dx=x \) days after the procedure, and \( d0= \) tumor volume two weeks after tumor implantation. The ratio of the caudal, non-embolized liver lobe (%CLV), was calculated according to the following formula:

\[
\%CLV = \frac{CLV \times 100}{(TLV-CLV)}
\]

Atrophy was calculated as: \( \% \) cranial liver lobe = \((TLV-CLV)-TV_{cranial}) \times 100 / TLV

**Wet-to-dry weight ratio**

Caudal and left lateral lobe biopsies were weighed after sacrifice (wet weight). The specimens were stored for 4 weeks in a stove at 60°C; hereafter the biopsies were weighed again (dry weight). The wet-to-dry weight ratio was calculated by the formula: (wet weight – dry weight) \times 100 / wet weight.

**Liver to body weight index**

The total liver and caudal liver lobes were weighed after sacrifice by means of a precision scale (Sartorius, Göttingen, Germany). The body weight can influence the total liver weight; therefore, caudal liver lobe weights were divided by the body weight.

**Mediators of liver regeneration**

The cytokines interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-\( \alpha \)), as well as the growth factors hepatic growth factor (HGF), and transforming growth factor beta 1 (TGF-\( \beta \)1) were determined in homogenized liver tissue of the left lateral and caudal liver lobe after sacrifice on day 14, using an ELISA kit for the respective antigen (USCN Life, Wuhan, China). All antibodies were washed out 4x with phosphate buffered saline (PBS, 1%BSA). Cytokines were assessed using polyclonal TNF-\( \alpha \) and IL-6 goat anti-rabbit antibodies (USCN Life, Wuhan, China). Measurements were repeated and concentrations were calculated from a standard curve. A BCA Protein Assay kit (Pierce, Rockford, IL) was used for evaluation of protein concentrations. All values were normalized to protein content.

**Biochemical assessments**

Liver function and damage parameters were assessed by routine clinical chemistry. Blood samples were obtained before tumor implantation, and after two weeks of tumor growth, before PVE or laparotomy. Three hours after the procedure, and on post-operative day 1, 3, 7, 10 and 14 blood samples were also obtained. AST (aspartate aminotransferase),
ALT (alanine aminotransferase), AP (alkaline phosphatase), γGT (gamma-glutamyl transpeptidase), and bilirubin were determined as liver damage parameters. Liver synthesis function was determined by measurements of prothrombin time and albumin.

**Histology**

In all groups, biopsies from the left lateral, embolized lobe and the caudal, non-embolized liver lobe were taken at sacrifice. Tissue samples were routinely fixed in 4% formalin (48 hours) and processed to paraffin tissue blocks. 4 μm sections were cut and stained with haematoxylin and eosin (H&E). H&E slides were blindly evaluated by an experienced liver pathologist.

Steatosis was estimated as the percentage of involved hepatocytes: grade 0 (absent; <5%), grade I (mild; 5-33%), grade II (moderate; 33-66%), or grade III (severe; >66%). Portal inflammation was arbitrarily graded as follows: 0 (absent), 1 (mild), 2 (moderate), or 3 (severe). Sinusoidal dilation was scored as: 0 (absent), 1 (mild; involving ≤ one-third of the (centro-)lobular area), 2 (moderate; involvement ≤ 2/3 of the parenchyma), or 3 (severe; involving ≥ 2/3 of the liver parenchyma). Intralobular inflammation and lytic necrosis were graded as: 0 (not present), 1 (<2 foci per x10 objective), 2 (2-4 foci per x10 objective), 3 (5-10 foci per x10 objective), and 4 (>10 foci per x10 objective). Portal edema was scored as the percentage of the portal tracts involved: 0 (not present), 1 (<25%), 2 (25-50%), 3 (50-75%), and 4 (>75%). The presence of areas with confluent necrosis of the parenchyma was scored as: 0 (absent), 1 (affecting <25% of the parenchyma), 2 (affecting 25-50% of the parenchyma), 3 (affecting 50-75% of the parenchyma), and 4 (affecting >75% of the parenchyma). The presence or absence of embolization material with concomitant giant cell reaction was also evaluated.

Cytokeratin 8+18 / Ki67 sequential alkaline phosphatase double staining was performed to evaluate hepatocyte proliferation in normal, non-tumorous liver parenchyma of the embolized cranial liver lobes and the non-embolized caudal liver lobe. The double staining combined a general hepatocyte marker cytokeratin 8/18, clone K8.8 + DC10 (Abcam, Cambridge, UK) stained in red (Vector Red, Vector Laboratories, Burlingame, CA, USA) with the proliferation marker Ki67 (mouse anti-rat, clone MIB5) (Dako, Glostrup, Denmark) in blue (Vector Blue) and a weak hematoxylin counterstain. Five multispectral data sets per case were acquired using a Nuance™ camera system (Caliper Life Science, Hopkinton, MA) from 420-720 nm at intervals of 20 nm. To analyze the percentage of Ki67 positive hepatocytes, spectral data sets were analyzed with the segmentation and machine-learning Inform™ 1.2 software (Caliper Life Science) similar to that described by Al-Kofahi et al. This software allows cell-by-cell analysis of all multispectral data sets for the co-expression of cytoplasmic cytokeratin 8/18 and nuclear Ki67 within all hepatocytes, thus excluding the proliferation of leucocytes and other cell types.

**Statistical analysis**

Data are tested for normal distribution, and equal variances. Values are expressed as means ± SD, unless otherwise stated. Differences in tumor volume, and CLV were analyzed by
using a repeated ANOVA model, and the Kruskal-Wallis test as appropriate. Furthermore, ANOVA with the linear mixed model for repeated measurements data was also performed for different liver biochemistry levels. The separate time points were analyzed by means of the two-tailed unpaired Student’s t-test for parametric continuous data. The Mann-Whitney U test was used for non-parametric data. Histological specimens were evaluated using the Fisher’s Exact test and Chi-square test where appropriate. Since most of the histology scores are ordinal (there is a ranking in the categories), the linear by linear association test was also used, which is identical to the Fisher’s Exact test. Statistical significance was accepted when \( p < 0.05 \). The data were analyzed by statistical software (SPSS 18.0.0; SPSS, Chicago, Illinois, USA) and GraphPad Prism (Graph-Pad Software, San Diego, CA).

Results

The VX2 cells were successfully implanted into the left medial liver lobes of all rabbits \((n=10)\). Following implantation, the fragments grew as one tumor, which enlarged rapidly. Two weeks after the implantation of the VX2 carcinoma in the liver, the solid tumor was clearly visualized on CT imaging, and could be used for the experiments.

Portal vein embolization

Portograms performed directly after embolization and at sacrifice showed complete occlusion of the portal vein branch to the cranial liver lobes in all PVE-rabbits \((n=5; \text{Figure 1})\).

Hypertrophy response of non-embolized caudal liver lobe

As expected, the volume increase of the caudal liver lobe was significantly higher in the PVE-group, compared to the control-group (Figure 2). An increase in CLV was seen until day 7, and only little additional increase was observed until day 10 and 14. When considering all measurement points, significant differences were found between the PVE- and control-group for the caudal lobes \((p<0.001)\). When the time points were analyzed one-by-one, significant differences were also seen from day 3 until day 14 \((p<0.01)\). Before PVE or laparotomy alone, there were no differences in %CLV between both groups.

In the PVE-group, the rate of Ki67 positive hepatocytes in the caudal liver lobe was higher (median 7.0, range 4.0-10.0%) than in the embolized, cranial liver lobes (median 0.3, range 0.1-1.2%; \( p=0.037 \)). In the control group, the caudal and cranial liver lobes showed the same amount of proliferating hepatocytes \((p=0.606)\). The rate of Ki67 positive hepatocytes in the caudal, non-embolized liver lobes in the PVE-group was higher (median 7.0, range 4.0-10.0%) compared to the caudal liver lobes of the control group (median 1.8, range 1.4-2.2%; \( p=0.083 \)) whereas a lower rate of Ki67 positive hepatocytes was found in the atrophic, embolized cranial liver lobes (median 0.3, range 0.1-1.2% vs control: median 1.4, range 0.8-8.2%; \( p=0.007 \)). The wet-to-dry weight ratios of liver biopsies were not significantly different between the groups (data not shown), excluding the possibility that edema was the cause of the volume increase.
The liver-to-body weight index of the caudal liver lobes at sacrifice were consistent with the CT volumetry data. The mean liver-to-body weight index of the caudal lobe of the PVE-group was 0.0118±0.0018 mL/g versus 0.0042±0.0001 mL/g (p=0.009) in the control group. No significant differences were found between or within both groups as regards the cytokines IL-6 and TNF-α, or the growth factors HGF and TGF-β1 in both cranial and caudal liver lobes (data not shown).

**Figure 1.** Portography in rabbits. Panel A shows a portogram before intervention. The portal vein to the caudal and cranial liver lobes is subsequently filled with contrast fluid. Panel B shows the hypervascular tumor (white arrow) before PVE. A radiographic image acquired 14 days after PVE is presented in panel C, in which the portal blood flow to the cranial liver lobes is completely occluded by particles and coils. The rabbit liver anatomy is schematically depicted in panel D (CL=caudal liver lobe, LL=left lateral liver lobe, LM=left medial liver lobe with VX2 carcinoma, and RL=right liver lobe). The grey line represents the level of PVE.

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**Tumor growth as measured by CT volumetry**

Tumor sizes two weeks after implantation (before starting the procedures) were different in both groups, with a mean volume of 2.50±0.5 mL in the control group, compared to a
volume of $0.58 \pm 0.1$ mL ($p=0.009$) in the PVE-group. Two weeks after PVE or laparotomy alone, tumor volumes were still larger in the control group ($p<0.01$). This is not surprising, since tumor growth is usually exponential. Therefore, a larger tumor load at the beginning of the analysis also resulted in a larger tumor load at the end. TGR is therefore, a better marker for tumor growth over time. Tumor growth rate (TGR) data are shown in figure 3. TGR was slightly increased in the first week, and further increased from day 7 until day 14 in both groups, showing significantly higher values in the PVE-group.

**Biochemistry**

The liver damage parameters AST and ALT were significantly higher in PVE-rabbits, with peak concentrations on day 1, remaining high until day 3, and declining thereafter (Figure 4). When considering all measurement points, no significant differences were seen in AST-levels between the PVE- and control-group ($p=0.07$), although a significant difference was observed for ALT ($p=0.013$). When we analyzed the time points separately, the AST values were significantly different as of 3 hours after PVE until day 7, and the ALT levels from day 1 until day 7. There were no differences in synthesis functions of the liver, as assessed by prothrombin time. Albumin was significantly decreased in the PVE group on day 3 (mean albumin PVE-group 39.6±1.1 vs 49.6±1.1g/L; $p=0.009$), and on day 7 (PVE:

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**Figure 2.** Volume increase rate of CLV as measured by CT volumetry, over 14 days after PVE. The mean %CLV is significantly higher in the PVE-group from day 3 until day 14 (*$p<0.01$), compared to the control group.

**Figure 3.** Tumor growth rate (TGR) following PVE/control. A larger increase was determined in the PVE-group over time with a mean TGR of 34.4±4.3 on day 14 in the PVE-group vs 24.1±7.2 ($p<0.05$) in the control group.
46.0±0.8g/L vs control: 48.6±0.4g/L, p=0.007). Hereafter, these values returned to normal. When considering all measurement points, there was no significant difference between the groups for albumin levels (p=0.097). On day 10, bilirubin-levels were higher in the PVE-group than in the control group (median PVE 2.0±0.5 vs control: 1.0±0.0; p=0.014). Over time there was also a significant difference in plasma bilirubin values (p=0.002).

Histology

No steatosis was found in any animal in cranial or caudal liver lobes. Portal and lobular inflammation, focal lytic necrosis, and portal edema were mild in both liver lobes in the two groups (NS, within or between groups). Mild or moderate sinusoidal dilation was seen in both groups in cranial and caudal liver lobes (NS, within or between groups) in terms of extent as reported by others in humans12; the width of the sinuses was however, not evaluated. Confluent necrosis of liver parenchyma in both groups was <25% (range 0-50%) for both cranial and caudal liver lobes (NS, within or between groups). A multinucleated giant cell reaction was observed around the portal vessels with the PVA particles in 4 out 5 embolized liver lobes, as evidence of a foreign body reaction.

![Liver damage parameters after PVE and control animals.](image)

Figure 4. Liver damage parameters after PVE and control animals. Plasma AST values showed a significant, transient increase with peak concentrations on day 1 following PVE in panel A (*p<0.05). ALT-values were also significantly elevated in the first week after PVE (#p<0.05), and returned to normal after 7 days (panel B).
Discussion

A small-for-size future remnant liver is an important reason why patients are considered unresectable. PVE is a treatment that is performed preoperatively to induce hypertrophy of the non-embolised FRL. Several clinical studies reported an increase in tumor growth after PVE in patients with primary or secondary liver tumors. The concerns that PVE potentially enhances tumor proliferation is confirmed by our study, in which TGR was significantly increased after PVE over time in a rabbit tumor model. However, there are no reports comparing tumor growth in patients with or without PVE with comparable tumor loads, before and after (the time of) PVE. In this study, we aimed to assess tumor progression before and after PVE, although there also is natural growth of tumor with the lapse of time. Therefore, a control group without PVE was included in our study.

Several animal studies reported changes in tumor volumes in embolized or ligated liver lobes as ascribed to upregulated cytokines, transcription factors, and regulatory factors. One of these studies showed that liver metastases in rats were smaller in embolized liver lobes (PVE) but larger in the ligated liver lobes (PVL), compared to sham laparotomy. Bretagnol et al. reported a decrease in tumor growth in the embolized lobe of rats after PVE. Another study performed in a mice tumor-model showed that tumor growth had doubled after PVL compared to 70% hepatectomy. These results are conflicting and furthermore, only models with small animals have been used. The rabbit liver is more suitable for the purpose of these studies, because PVE can be performed easily in a liver tumor model in which the caudal liver lobe accounting for approximately 20% of total liver volume, is isolated from the embolized, cranial liver lobes. Furthermore, CT-scans were used for determination of CT volumetry in rabbits, which resembles the clinical situation. The isolated, caudal liver lobe in the rabbit is easily distinguishable from the rest of the liver on CT-scan, which makes the model more appropriate than rat- or mice-models.

The VX2 celline was used for the experimental model in this study, having several advantages. The VX2 carcinoma is a rapidly growing, hypervascular tumor, mainly vascularized by the hepatic artery, similar as to human liver tumors. The tumor becomes large enough to be evaluated on CT images, and therefore, provides a perfect tumor model for our experiments. Moreover, the tumor is implanted into the subcapsular area of the liver, whereas in most rat and mice tumor models, the tumor cells are injected into the spleen, which may lead to immunological bias. Our model very much resembles the clinical setting of PVE. The cranial liver lobes corresponding to 80% of total liver volume are embolized, while the caudal, non-embolized liver lobe consists of only 20% of the total volume of the liver, which mirrors the situation in patients in whom the FRL is considered small-for-size.

TGR in our study was highest in the second week after PVE, however, this finding is clinically less relevant. In humans, the volume increase rate of the FRL is highest during the first 3 weeks following PVE, after which it plateaus. Liver resection is often planned from three weeks after PVE for this reason. The hypertrophy response in the rabbit model is
highest at day 7 with only little additional increase by day 10 and 14\(^3\), suggesting that the regenerative process after PVE is more efficient in rabbits.

There are some limitations to this study. CT volumetry was used to measure volumes of the total liver, the non-embolized liver lobe, and tumor. As mentioned, tumors were small two weeks after implantation (between 0.6 and 2.5 mL), which could introduce a bias when delineating the contours on each serial section of CT scans. However, the images were enlarged during delineation, rendering the outcomes more accurate. Furthermore, CT images were analysed by two experienced, independent researchers in a blinded fashion. TGR could however, not be determined before PVE since the tumors were not visible on CT scan on the day of tumor implantation.

As mentioned above, tumor volumes after two weeks of implantation were significantly higher in the control-group compared to PVE-rabbits (2.5±0.5mL vs 0.58±0.1mL, resp.). There are several explanations for this different outcome. Firstly, smaller tumor fragments may have been implanted in the PVE-rabbits, although we tried to avoid implanting different fragment sizes. Secondly, tumor growth could have been more rapid in the two weeks before intervention in the control group, despite the same VX2 celline was used and, laboratory conditions and operations were standardized. We assume that growth of the experimental tumor is exponential, resulting in a larger tumor load at the end when the tumor is also larger at the beginning of the analysis. Tumor sizes were possibly different between groups after initial injection of the tumor fragments. We therefore used TGR as marker for tumor growth, since these outcomes were compared to baseline values, and therefore, were more reliable.

Regarding the pathology results, no major differences were found in terms of steatosis, portal and lobular inflammation, focal lytic necrosis, portal edema, the extent of sinusoidal dilation or confluent necrosis, the latter being absent or mild in both lobes in both groups. The groups might have been too small however, to detect statistically significant differences, although the findings are in agreement with the transient, minimal increase in liver damage parameters found after PVE. In the embolized lobes, a giant cell reaction was found in association with embolization material, but this could not be demonstrated in one animal, probably due to a sampling error since effective embolization of the cranial lobe was evident at imaging. No differences were observed in cytokines or growth factors in the liver parenchyma between the PVE- and control-group at sacrifice. This is probably due to the cytokine responses which peak earlier in rabbits and therefore, were missed in this study. Along the same lines, growth factors released early after PVE, were possibly depleted after 14 days, showing no increase at that time anymore.

In conclusion, TGR was significantly increased over time in the PVE rabbit tumor model, supporting the notion that PVE potentially enhances tumor growth along with regeneration of the non-embolized liver lobe. Therefore, new interventions should be directed to preventing tumor growth after PVE.
Acknowledgement

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References