Optimizing preoperative portal vein embolization for liver resection

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CHAPTER 1

A review of animal models for portal vein embolization

F. Huisman, K.P. van Lienden, S. Damude, L.T. Hoekstra, T.M. van Gulik
ABSTRACT

Background:
Portal vein embolization (PVE) is a preoperative intervention to increase the future remnant liver (FRL) through regeneration of the non-embolized liver lobes. This review assesses all the relevant animal models of PVE available, to guide researchers who intend to study PVE.

Materials and methods:
We performed a systematic literature search in Medline and Pubmed, from 1993-June 2013, using search headings “PVE” and “portal vein ligation”. Articles were included when meeting the selection criteria: experimental animal study on PVE or portal vein ligation and experiments described in 5 animals or more.

Results:
Sixty-one articles were selected, describing six different animal models. Most articles reported experiments with rats, rabbits, and pigs. In rats, the increase in wet-weight ratio of the non-occluded liver or total liver weight is greatest in the first 7 d with values ranging from 75%-80.5% on day 7. The volume increase of FRL in the rabbit model is greatest in the first 7 d with values ranging from 33.6%-80% on day 7. In pigs, the largest gain in volume of the FRL was seen in the first 2 wk.

Conclusions:
The choice of the model depends on the specific aim of the study. Evaluating the increase in liver volume and liver function after PVE, larger animals as the pig, rabbit, or the dog is useful because of the possibility to apply computed tomography volumetry. To evaluate mechanisms of regeneration after PVE, the rat model is useful, because of the variety of antibodies commercially available.
BACKGROUND

Surgical resection of primary or secondary tumors in the liver remains the only curative therapy. The great majority of patients are however, not candidates for surgery because of tumor burden or too small liver remnant leading to increased risk of post hepatectomy liver failure. To undergo a major liver resection, the future remnant liver volume in humans has to be at least 25% based on CT volumetric studies to avoid post resectional liver failure [1]. In livers with compromised parenchyma due to cirrhosis, steatosis or recent chemotherapy, the minimum volume should be at least 30%. Preoperative portal vein occlusion by embolization or ligation is a method to stimulate growth of the non-occluded liver segments, thereby increasing the volume of the future remnant liver [2].

The concept of the atrophy-hypertrophy complex following unilateral portal vein occlusion has initially been demonstrated in a rabbit model by Rous and Larimore in 1920 [3]. They discovered in rabbits that ligation of the portal branches to part of the liver caused atrophy of that part of the liver and concomitant hypertrophy of the non-ligated part of the liver. This phenomenon has already been used in a clinical setting for many years. Although clinical PVE has shown effective, several issues need further investigation. The mechanism of induction of liver regeneration after PVE is still poorly understood, the optimal technique and choice of embolization materials can be improved and (pharmaceutical) interventions to stimulate liver regeneration in addition to PVE must be further investigated. As a downside, PVE not only induces liver regeneration, but it also promotes tumor growth [2, 4, 5]. Strategies to control this potential drawback need to be explored in animal studies. In this review, we describe all relevant animal models, used to study PVE, in relation to the species-specific anatomy techniques and the induced hypertrophy response.

MATERIALS AND METHODS

We performed a systematic literature search in Medline and Pubmed, from 1993 to June 2013. The applied search headings were: “portal vein embolization” and “portal vein ligation”. Limitations were set to English language and animal studies. The abstracts were screened to identify potentially relevant articles and were evaluated by two of the authors (FH, SD, LH), using a predetermined scoring list. Full text articles of potentially relevant papers were screened and were included in this study when meeting the following selection criteria:
- Experimental animal study on PVE or PVL
- Experiments described in at least 5 animals.
CHAPTER 1

RESULTS

Types of animals

Sixty-one articles were selected, describing 6 different animal models; i.e. in monkeys, pigs, dogs, rabbits, rats or mice (Table 1). One article discussed both dogs and rats. Two articles described both PVL and PVE in a rat model, rabbit model and pig model.

Table 1. Animal models used for PVE.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Number of articles PVL</th>
<th>Number articles PVE</th>
<th>Total number of articles</th>
</tr>
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<tbody>
<tr>
<td>Monkey</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pig</td>
<td>5</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Dog</td>
<td>1</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Rabbit</td>
<td>2</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Rat</td>
<td>20</td>
<td>8</td>
<td>27</td>
</tr>
<tr>
<td>Mouse</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

Mice

Only three articles described the procedure of PVL in a murine model and no reports of PVE in the mouse have been published [6-8]. The diameter of the portal branches of the mouse is small; therefore it is not an ideal animal model to perform PVE.

Monkeys

One article described the use of a monkey model to observe the regeneration response after PVE with an absorbable embolization material [9]. The use of monkeys for research is in many countries restricted or forbidden.

Rats

Twenty-seven articles described PVE or PVL in a rat model.

Anatomy of the rat liver

The liver of the rat consists of four lobes. The left part of the liver part consists of the median lobe and the left lateral lobe. The right part of the liver consists of the superior and inferior right lobe, the anterior and posterior caudate lobe. Each lobe has its own blood supply consisting of branches of the portal vein and hepatic artery (Figure 1).

Technical procedure in the rat

In 7 of the 8 articles describing PVE in rats, the left liver lobe was embolized, corresponding to 70% of total liver volume (Figure 2; [10-16]). The main portal trunk was dissected and punctured with a needle ranging from 20 – 30-gauge and a catheter was connected through
A review of animal models for portal vein embolization

which portography was performed. The tip of the catheter was placed just above the right portal branches and transcatheter embolization was possible. Selective embolization is done by temporary clamping of the portal branches of the liver lobes to be preserved.

Furrer et al. chose to embolize the right liver lobes and the left lateral lobe, leaving only the portal branch to the median lobe open (Figure 3; [17]).

Figure 1 Anatomy of the rat liver.

Figure 2 Schematic view of the rat liver lobes showing occlusion of the portal vein branches to the left liver lobe, i.e., the median lobe (ML) and the left lateral lobe (LLL), and preservation of the right liver, i.e., the superior (SRL) and the inferior right lobe (IRL), the caudate process (CP), the anterior (AC) and posterior caudate lobe (PC).
Volume increase of future remnant liver and time to maximum hypertrophy

In one study, rats were sacrificed after 4 days. Most studies, however, chose to sacrifice after one week (9 out of 27 studies), or after 2 weeks (8 studies). Accelerated growth of the non-embolized liver was seen in the first 3-4 days after which the hypertrophy response subsequently decreased. In none of the studies, did liver regeneration reach a plateau phase at the time of sacrifice, suggesting that rats should be observed at least 14 days to fully appreciate the regenerative capacity.

To evaluate the hypertrophy response after PVE or PVL in rats, weights of the non-occluded parts of the liver have been measured at sacrifice and volume increase was calculated using the following formulas:

- Weight of the non-embolized liver or non-ligated liver lobes / total liver weight (%) (Table 2).
- Weight of the non-embolized liver or non-ligated liver lobes / body weight (%) (Table 3).

The increase of wet weight ratio of the non-occluded liver / total liver weight is greatest in the first 7 days with values ranging from 75 to 80.5% on day 7. In two studies, rats were sacrificed on day 14 and 28, with an ultimate increase in volume of 80% and 95%, respectively.

Rabbits

Anatomy

The liver of the rabbit consists of four main lobes: one caudal liver lobe and three cranial liver lobes (Figure 4). The caudal liver lobe is separated from the cranial liver lobes and therefore, can be clearly distinguished.
### Table 2. Ratio of the wet weight of non-occluded lobes of the liver after PVL/PVE / total liver (%).
(* estimation of the increase found in graphs in the articles)

<table>
<thead>
<tr>
<th>PVE/PVL</th>
<th>Author</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVL</td>
<td>Schweizer et al. [18]</td>
<td>38* %</td>
<td></td>
<td>60*</td>
<td></td>
<td>75*</td>
<td></td>
<td></td>
<td>95*</td>
<td></td>
</tr>
<tr>
<td>PVL</td>
<td>Vetelainen et al. [19]</td>
<td></td>
<td>50*</td>
<td></td>
<td>60*</td>
<td></td>
<td></td>
<td>80*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVL</td>
<td>Yu et al. [20]</td>
<td>38.39±2.25 (SD)</td>
<td>60.02±4.22 (SD)</td>
<td></td>
<td></td>
<td>77.68±2.34 (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVL</td>
<td>Mizuno et al. [21]</td>
<td>30±2(SD)</td>
<td></td>
<td></td>
<td></td>
<td>80±1(SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVL</td>
<td>Iuchi et al. [11]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>63.1±3.5 (SD)/59.9±2.7 (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVL</td>
<td>Kucuktulu et al. [25]</td>
<td>1.4±0.1(SD)</td>
<td></td>
<td>1.8±0.1(SD)</td>
<td></td>
<td>3.0±0.2(SD)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>PVE/PVL</td>
<td>Lee et al. [12]</td>
<td>1.0*/1.0*</td>
<td></td>
<td>1.2*/1.7*</td>
<td></td>
<td>1.8*/2.5*</td>
<td></td>
<td>2.8*/2.8*</td>
<td>3.1*/3.0*</td>
<td></td>
</tr>
<tr>
<td>PVL</td>
<td>Tanaka et al. [16]</td>
<td>1.39±0.05(SD)</td>
<td></td>
<td>2.0*</td>
<td></td>
<td>2.6*</td>
<td></td>
<td>2.89±0.05(SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVL</td>
<td>Uemura et al. [26]</td>
<td>1.2*</td>
<td>1.4*</td>
<td>1.8*</td>
<td>1.9*</td>
<td>2.5*</td>
<td>2.4*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVL</td>
<td>Kong et al. [27]</td>
<td>1.9*</td>
<td>2.2*</td>
<td>2.8*</td>
<td>3.2*</td>
<td>3.8*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVL</td>
<td>Makino et al. [28]</td>
<td>1.4*</td>
<td>1.8*</td>
<td></td>
<td>2.8*</td>
<td></td>
<td>2.9*</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PVL</td>
<td>Sugimoto et al. [23]</td>
<td>0.39±0.04(SD)</td>
<td></td>
<td></td>
<td></td>
<td>0.86±0.07(SD)</td>
<td>1.03±0.05(SD)</td>
<td></td>
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<tr>
<td>PVL</td>
<td>Morine et al. [24]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.802±0.09(SD)</td>
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</tbody>
</table>

### Table 3. Ratio of the non-embolized liver or non-ligated liver lobes / body weight. (%)

<table>
<thead>
<tr>
<th>PVE/PVL</th>
<th>Author</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVL</td>
<td>Kucuktulu et al. [25]</td>
<td>1.4±0.1(SD)</td>
<td></td>
<td>1.8±0.1(SD)</td>
<td></td>
<td>3.0±0.2(SD)</td>
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</tr>
<tr>
<td>PVE/PVL</td>
<td>Lee et al. [12]</td>
<td>1.0*/1.0*</td>
<td></td>
<td>1.2*/1.7*</td>
<td></td>
<td>1.8*/2.5*</td>
<td></td>
<td>2.8*/2.8*</td>
<td>3.1*/3.0*</td>
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</tr>
<tr>
<td>PVL</td>
<td>Tanaka et al. [16]</td>
<td>1.39±0.05(SD)</td>
<td></td>
<td>2.0*</td>
<td></td>
<td>2.6*</td>
<td></td>
<td>2.89±0.05(SD)</td>
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<td></td>
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<tr>
<td>PVL</td>
<td>Uemura et al. [26]</td>
<td>1.2*</td>
<td>1.4*</td>
<td>1.8*</td>
<td>1.9*</td>
<td>2.5*</td>
<td>2.4*</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PVL</td>
<td>Kong et al. [27]</td>
<td>1.9*</td>
<td>2.2*</td>
<td>2.8*</td>
<td>3.2*</td>
<td>3.8*</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PVL</td>
<td>Makino et al. [28]</td>
<td>1.4*</td>
<td>1.8*</td>
<td></td>
<td>2.8*</td>
<td></td>
<td>2.9*</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PVL</td>
<td>Sugimoto et al. [23]</td>
<td>0.39±0.04(SD)</td>
<td></td>
<td></td>
<td></td>
<td>0.86±0.07(SD)</td>
<td>1.03±0.05(SD)</td>
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<tr>
<td>PVL</td>
<td>Morine et al. [24]</td>
<td></td>
<td></td>
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<td></td>
<td>0.802±0.09(SD)</td>
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</table>

(* estimation of the increase found in graphs in the articles)
Technical procedure in the rabbit

In 6 of 8 articles, PVE was performed by embolizing the portal branches to the cranial liver lobes. These lobes account for 80% of total liver volume. After a midline laparotomy, the mesenteric vein is cannulated and via a 3 French microcatheter, the portal branches to the cranial liver lobes are embolized [5, 18-22]. In two articles, a different method to embolize a part of the rabbit liver was performed. The authors occluded the external left branch of the portal vein, supplying the left lateral lobe (accounts for 25% of the total liver volume) [23, 24]. A percutaneous transhepatic puncture of the external left branch of the portal vein was undertaken to avoid laparotomy. Besides PVE, the rabbit is an excellent model to perform other procedures including arterial and hepatic venous embolization [22].

Volume increase of future remnant liver and time to maximum hypertrophy

As shown in Table 4, the regeneration response reaches a plateau-phase already after 7 days, allowing experiments with short observation-time (Figure 5).

CT volumetry is a very suitable method to measure the growth of the non-occluded liver non-invasively, similar to the assessment of future remnant liver in patients after PVE. All authors used CT volumetry, which enables repeated measurements within one rabbit. The caudal, non-embolized liver lobe (CLV) can easily be delineated and measured. The increase in CLV is measured with the following formula:

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

The volume increase of the future remnant liver weight is greatest in the first 7 days with values ranging from 33.6% to 80% on day 7. The maximum observation time after PVE reported in rabbits is two weeks.
A review of animal models for portal vein embolization

Dogs

PVE in dogs was described in 7 articles and 1 article described PVL.

Anatomy

The liver of the dog consists of seven lobes; the left portal branch perfuses the left lateral lobe and papillary process, the left medial lobe, the quadrate lobe and the right medial lobe.

Table 4. Increase in FRL (%) after PVE in the rabbit model

<table>
<thead>
<tr>
<th>PVE/PVL</th>
<th>Author</th>
<th>Day 3</th>
<th>Day 7</th>
<th>Day 10</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVE</td>
<td>De Graaf et al. [29]</td>
<td>38*</td>
<td>80*</td>
<td>85*</td>
<td>88*</td>
</tr>
<tr>
<td>PVE</td>
<td>Van den Esschert et al. [32]</td>
<td>33.6±10</td>
<td>79.8±18.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVE</td>
<td>Van Lienden et al. [33]</td>
<td>33.6±4</td>
<td>79.8±8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HVE</td>
<td>-10.2±5.5</td>
<td>5.6±7.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HVE+PVE</td>
<td>43.4±7.5</td>
<td>103.6±10.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(* estimation of the increase extracted from graphs in the articles)

Figure 5 Increase in CLV measured by CT volumetry in the rabbit PVE model.

Figure 6 Anatomy of the liver of the dog. LL = left lateral lobe, PPC = papillary process of caudate lobe, LM = left medial lobe, QL = quadrate lobe, RM = right medial lobe, CPC = caudate process of caudate lobe.
The right portal branch perfuses the right lateral lobe and caudate process (Figure 6). In dogs, the left liver lobe corresponds to 70% of total liver volume [25].

**Technical procedure**

The most frequently used procedure to perform PVE in dogs was through laparotomy and direct injection of an embolization material into the left branch of the portal vein under angiographic guidance [26-28]. The materials used were hydrophilic phosphorylcholine, n-butyl cyanoacrylate, hydrophilic gel, polyvinyl alcohol (PVA) particles, polidonacol and gelatine sponge, steel coils, absolute ethanol, gelfoam with and without coils (Figure 7).

One of the articles described a procedure of PVL in the dog model in which left portal branch ligation was performed in 23 dogs [27].

![Figure 7 Schematic representation of portal vein embolization in a dog liver. LL = left lateral lobe, PPC = papillary process of caudate lobe, LM = left medial lobe, QL = quadrate lobe, RM = right medial lobe, CPC = caudate process of caudate lobe.](image)

**Volume increase of future remnant liver and time to maximum hypertrophy**

Various methods to evaluate the hypertrophy response have been used in dogs making it difficult to compare the results described in different articles. Huang et al. measured the hypertrophy response using CT volumetry and showed that regeneration had reached a plateau phase after 6 weeks, suggesting that dogs should be sacrificed after at least 6 weeks following PVE [26]. Kaneko et al. evaluated hepatic regeneration by measuring the relative liver weight ratio (LWR): (Non-embolized liver weight / embolized liver weight) x 100%. They occluded the portal vein branches to the right medial, quadrate, left lateral, and papillary process of caudate lobe. During an 8 weeks waiting period, the LWR increased from 31.8%
in control animals to 220.8% after embolization. They concluded that in dogs the atrophy/hypertrophy response continued for 8 weeks after embolization [29].

**Pigs**

The pig model has been used in 5 articles concerning PVL and 9 articles concerning PVE.

**Anatomy**

The pig liver consists of 4 lobes, i.e. the right lobe, right middle lobe, left middle lobe and left lobe. The lobar volume distribution of the porcine liver is similar to human livers (Figure 8). The left lateral segments account for 20-25% of total liver volume.  

![Figure 8 Hepatic lobar anatomy of a pig liver, consisting of the right lobe (R), right middle lobe (RM), left middle lobe (LM) and left lobe (L).](image)

**Technical procedure**

Nine articles described a pig model for PVE. The most common method to perform embolization of the left portal vein branch perfusing the left middle lobe and left lobe was by percutaneous transhepatic approach [30-36]. Smits et al. tried to find a less invasive method to embolize the right lateral and medial lobe (n=6) together with the left lateral and medial lobe of the porcine liver. They demonstrated that portal vein embolization can be performed by a new technique called retrograde transsinusoidal injection of low-viscosity liquid embolic agent [37]. This technique is based on the phenomenon that contrast fluid is able to pass in retrograde fashion through the sinusoid and reach the portal vein. The authors were the first to demonstrate that PVE can be accomplished by retrograde injection of the embolic agent from a wedged catheter in the hepatic vein. The hypertrophy response was however, not assessed. Madoff et al. have also attempted to find a less invasive approach to perform PVE [32]. They demonstrated another, indirect method to occlude the portal venous system by the transarterial approach. This approach may minimize complications and make the procedure easier. Transarterial PVE
was compared with transhepatic, transportal PVE. For transarterial PVE, the femoral artery was cannulated and a catheter was advanced into the hepatic arterial branches supplying the left and left middle liver lobes. The embolization material was injected across the peribiliary arterioporal plexus into the portal veins resulting in occlusion of the portal venous system. They concluded that transarterial portal vein embolization is safe and effective for inducing liver regeneration in the swine. In most of the articles, the left and median lobes were embolized.

*Volume increase of future remnant liver and time to maximum hypertrophy*

Due to the lack of reporting standards for PVE in the pig, no conclusions can be drawn from these articles regarding volume increase of the future remnant liver.

The time from PVE to sacrifice was 1-6 weeks. Park et al. found, as a result of embolization with Embol-78, that the volume ratio of the non-embolized liver changed from 55% to 71% at 2 weeks and to 84% at four weeks [33]. This was determined by weighting the liver lobes after sacrificing the pigs. Satake et al. measured a mean future remnant liver / embolized liver volume ratio increase after PVE with absolute ethanol, measured by CT-volumetry after 3 weeks, of 14.2%. The largest gain in volume of the future remnant liver was seen in the first two weeks.

The most frequently used method to evaluate the future remnant liver volume was CT-volumetry [32, 34, 35]. The peak of regeneration in pigs after PVE or hepatectomy is described to occur at 7 days [31].

*Embolization materials*

Since the first article in 1986 has been published by Kinoshita et al. [38] reporting the use of PVE in patients, various embolization materials have been used to perform PVE. No randomized trials have been performed evaluating the efficacy of the different embolization materials in inducing hypertrophy. Only one retrospective clinical study compared N-butyl cyanoacrylate (NBCA) and Polyvinyl alcohol (PVA) micro particles with coils, concluding that the use of NBCA induces a greater regeneration response of the FRL [39]. Table 5 shows the various embolization materials that have been used in the animal models described above.

It is not clear which embolization material shows the best results. The embolization materials mentioned are often used in combination and each experiment used its own standard, precluding conclusions on which material is most effective in inducing a hypertrophy response of the FRL.

*Tumor models*

Besides regeneration of the FRL, several studies describe potential tumor progression after PVE, which is a serious drawback of this technique [4, 40, 41]. Animal tumor models have been used to investigate enhanced tumor growth after PVE.
A review of animal models for portal vein embolization

Qi et al. [23] were the first who used a VX2-tumor model in rabbits in combination with portal vein ligation to investigate the effect on tumor growth. The VX2-tumor used was derived from a virus-induced papilloma tumor in rabbits. Zou et al. [24] were the first using the VX2-tumor model in combination with PVE. The tumor is of non-hepatic origin, but grows rapidly and the blood supply is similar to human hepatocellular carcinoma. Therefore, it is a very well suited tumor model for evaluating the influence of PVE on tumor growth.

To investigate the effect of PVE on tumor growth, the rabbit model was used in two articles [5, 24]. The VX2-tumor cells were implanted in the hind limb of the donor rabbit. After three weeks, the tumor cells were collected and cut into tumor fragments. The fragments were directly injected superficially under the liver capsula in the cranial lobes and PVE was performed two weeks later. In both reports, the authors concluded that PVE promotes tumor growth and that the rabbit model is an ideal model to use because of the isolated, non-embolized caudal liver lobe allowing selective implantation of tumor.

In the rat model, three articles investigated the differences in tumoral responses in the liver after PVL and PVE. Bretagnol et al. found a decrease in liver metastases in the embolized liver after injecting the tumor cells in the left medial liver lobe. Iuchi et al. injected tumor cells in the portal vein to produce liver metastasis on both sides of the liver. They found a significant reduction of tumor growth in the non-embolized lobes after embolization [10, 11, 14]. The other study by Maggiori et al. however, showed an opposite effect of PVL and PVE on tumor growth, in which PVL and PVE both increased tumor growth in both the

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embolized and non-embolized liver lobes.[14] The tumor cells used in the latter two rat models were DHD/K12 cells, which were directly injected under the liver capsule. DHD/K12 is an established, transplantable colon carcinoma cell line. This tumor model in rats has the advantage that the DHD/K12 cells provide the biologically most accurate in vivo model of development of early colonic liver metastasis [42].

**DISCUSSION**

In this article we give an overview of the relevant literature on animal models used to investigate the hypertrophy response after PVE, in order to guide researchers who intend to do research on PVE.

Over the years, several animal models of PVL and PVE have been used describing different techniques, in most instances specific for the animal species used. Every animal model obviously has its own advantages and disadvantages.

The rat model is the most common model for PVE. This is the fastest model with lowest costs. Rabbits are more expensive, but they are easy to handle and to house. Experiments with pigs are labour intensive, expensive and not every research center has facilities to accommodate these pigs. Dogs are the most expensive animals and their use for research purposes is controversial in Europe. Housing facilities are also expensive for dogs.

Concerning the technical approach, in humans, three different techniques to perform PVE have been described, i.e. trans-ileocolic, contralateral and ipsilateral. These techniques have also been described in the animal models. Madoff et al. demonstrated a new, transarterial approach to embolize the portal branches in a pig model [32]. Absolute ethanol was infused through a microcatheter via the hepatic artery braches reaching the portal system via the peribiliary plexus. No adverse events were seen and the hypertrophy response of the FRL was comparable to the transhepatic approach. There are no reports showing applicability of this approach in other animal species or in humans.

Experimental studies and series in humans comparing PVE and PVL showed conflicting results regarding outcomes of the regeneration response. This issue has also been studied in rats, rabbits, dogs and pigs. In the pig model, only one study by Wilms et al. concluded that PVE is more effective than PVL to induce a hypertrophy response [36]. In the rabbit, Van den Esschert et al. found that PVE is superior to PVL in terms of the regeneration response [20]. This may be due to the fact that after PVL, formation of collateral portal vessels leads to portal reperfusion of the parenchyma distal to the ligature. This phenomenon has also been demonstrated in humans as published by Van Lienden et al. The authors visualized on fluoroscopy 3 weeks after PVE, the formation of intrahepatic portoportal, neocollateral vessels reperfusing the ligated lobe, which made them conclude that PVL is less effective in inducing a hypertrophy response of the nonligated lobe [43].
In contrast, Lee et al. found that PVE had the same effect as PVL on DNA synthesis and cell proliferation in rats [12]. They also concluded that the increase in volume of the future remnant liver lobes was higher after PVL compared to the PVE group. Furrer et al., also using rats, concluded that PVL is superior to PVE in inducing a regenerative response of the non-occluded liver [17]. This was thought to be due to a foreign body reaction induced by the embolization material used with PVE leading to less blood flow and less macrophages involved in the regenerating part of the liver.

The rat is much smaller than the human being and experimental results therefore, are less translatable to the clinical situation. The porcine liver is suitable for PVE because of the liver anatomy similar to the liver lobes in humans. For comparative studies, the pig model would be most appropriate because of the large calibre of the portal vein branches.

The mean diameter of the portal vein branches of the rat is too small to apply coils and large embolization particles. PVE in rats and mice is very difficult because of the small size of the veins of the portal system resulting in a high failure rate.

The rabbit provides a unique model, because of the anatomy of the liver. The separated, cranial liver lobe can be readily embolized while hypertrophy of the non-embolized, caudal liver lobe can be accurately monitored by CT-scans.

CT volumetry is used in humans to evaluate the hypertrophy response expressed as a volume increase after PVE. This technique is also often used in the rabbit and the pig model. In rabbits, an accurate measurement of the hypertrophy rate of the non-embolized liver lobes can easily be performed by sequential CT-volumetry on several time points, without sacrificing the animals. This is also possible for the pig, however requires special facilities. The rat liver is small and the resolution of the CT-scan is too low to obtain accurate estimation of the increase in volume.

Another method of assessing the response after selective PVE is to determine functional increase of the non-embolized liver lobes. We found 3 articles in which 99mTc- diisopropyl iminodiacetic acid (DISIDA) dynamic SPECT (single-photon emission computed tomography) was used to measure liver function in a rat model [44-46]. Lin et al. evaluated the functional changes after PVL in cirrhotic and noncirrhotic rats [45]. They concluded that the regenerated, functional liver mass in cirrhotic rats after PVL was less than in non-cirrhotic rats. Tseng et al. used SPECT/CT only to determine liver volume instead of function in a rat model [47].

Rat liver samples can be easily used for screening of gene expression using DNA microarray techniques. This technique allows assessment of the cytokines and growth factors involved in the mechanism of regeneration after PVE. A disadvantage of the rabbit model as compared to the rat model is the lack of available antibodies to determine specific growth factors and cytokines induced by PVE by ELISA or immunohistochemical staining. This also applies to the pig and the dog model.

In conclusion, several animal models are available to study PVE, all with their own advantages and limitations. The choice of the model depends on the purpose of the experiment.
Evaluating increase in liver volume and liver function after PVE, larger animals as the pig, rabbit or the dog are useful because of the possibility to apply CT volumetry. To evaluate underlying mechanisms of regeneration (cytokines or growth factors) after PVE, the rat model is more useful, because of the variety of antibodies commercially available.

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References


A review of animal models for portal vein embolization


