Clinical and experimental studies on portal vein embolization / Diagnosis of hepatocellular adenoma and focal nodular hyperplasia

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Short-term effects of combined hepatic vein embolization and portal vein embolization on the induction of liver regeneration

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Abstract

Background: Because liver tumors may become irresectable in the waiting time between portal vein embolization (PVE) and liver surgery, alternative methods to optimize the hypertrophy response after PVE, particularly in terms of inducing maximum liver regeneration in minimum time, are desired. The aim of this study was to assess the effect of hepatic vein embolization (HVE) in addition to PVE on the liver hypertrophy response in a standardized rabbit model.

Materials and methods: Thirty New Zealand white female rabbits were allocated to a group according to the intervention: PVE alone, HVE alone, and a combination of HVE and PVE. Occlusion of the veins was assessed by a portogram and a contrast enhanced CT scan. The liver regeneration response of the caudal liver was assessed by CT volumetry, liver to body weight index, and the amount of proliferating hepatocytes.

Results: The caudal liver volume (CLV) increased significantly more in the PVE and combined PVE/HVE group compared to the HVE group, 3 and 7 days after the procedure (p<0.01). There were no significant differences in CLV increase or degree of hypertrophy between the PVE and the combined group. The caudal liver to body weight index was significantly higher in the PVE and combined group compared to the HVE group on day 7 (p<0.01). However, the index was also significantly higher in the combined PVE/HVE group compared to the PVE group (p=0.008). The caudal liver tissue of the PVE and combined group contained a significantly higher number of proliferating hepatocytes compared to the HVE group on day 7 (p<0.01).

Conclusion: The results of this study suggest that although histological and additional regenerative changes are seen, HVE in addition to PVE, has no significant effect on the hypertrophy response. The combination of HVE and PVE may therefore, have little use in a clinical setting.
Introduction

Since the first clinical application 25 years ago,\textsuperscript{1} portal vein embolization (PVE) has become a widely used method to increase the future remnant liver (FRL) before major liver resection.\textsuperscript{2} PVE is considered when the FRL is considered too small, thereby increasing the risk of postoperative liver failure.\textsuperscript{3} With PVE, the portal vein branches of the to-be-resected liver lobe are occluded, causing atrophy of this liver lobe. This results in a release of regenerative factors that in turn induces a compensatory hypertrophy response in the contralateral, non-embolized liver segments.\textsuperscript{4-6} In most cases, the hypertrophy response following PVE reaches its plateau after 21 days.\textsuperscript{7} Thereafter, only little additional growth takes place. When the FRL volume, typically measured by CT volumetry 3 weeks after PVE, is $\geq 25\%$ of the original total liver volume, partial liver resection is performed. However, the hypertrophy response is not always sufficient by this time. A drawback of PVE is the potential induction of tumor growth after PVE, leading to secondary irresectability.\textsuperscript{8} Therefore, much research effort is devoted to find alternative methods to optimize the hypertrophy response after PVE, particularly in terms of inducing maximum liver regeneration in minimum time.

Recently, a Chinese research group published on sequential, ipsilateral hepatic vein embolization (HVE) after PVE.\textsuperscript{9,10} In 12 patients who showed insufficient increase in volume of the FRL after PVE, the right hepatic vein was also embolized to induce additional hypertrophy. The additional HVE was performed 13.5 $\pm$ 4.2 days after PVE. Embolizing the ipsilateral hepatic vein blocks the hepatic outflow and, in combination with PVE, should decrease compensatory arterial hyperperfusion, thereby inducing additional hypertrophy of the non-embolized FRL. However, in this study, the increase in FRL could still be the result of ongoing hypertrophy caused by PVE alone. The hypertrophy response namely continues for at least 3 weeks in humans, before a plateau phase is reached. However, these findings conceived the idea that simultaneous PVE and HVE could possibly result in a greater hypertrophy response than PVE alone within the same time frame. To eliminate the influence of the time factor after PVE and to achieve the maximum hypertrophy result in a short follow-up period, we performed the PVE and HVE in one single procedure instead of sequentially. The aim of this study was to assess the effect of HVE in addition to PVE on the liver hypertrophy response in a standardized rabbit model.

Materials and methods

Animals

Experimental protocols were approved by the institute’s animal ethics and welfare committee. A total of 30 female New Zealand White rabbits (Harlan, Gannat, France) with a mean weight of 2,884 g (range 2,470-3,430 g) were acclimatized for 2 weeks under standardized laboratory conditions.
conditions in a temperature-controlled room with a 12-h light/dark cycle and access to standard chow and water ad libitum.

![Anatomy of the rabbit liver with the portal vein (A). Panel B shows a portogram after PVE, the coils are situated in the main portal vein branch to the cranial liver lobes (white arrow). The coils of additional HVE are visible on the portogram in panel C (black arrowhead).](image)

**Figure 1.**

**Experimental design**

A validated rabbit model was used for this study. The rabbit liver anatomy differs slightly from the human. It has 4 main lobes of which 3 are located cranially and 1 caudally. The 3 cranial lobes, which account for approximately 80% of the total liver, were embolized. The caudal lobe was spared and used as FRL to evaluate the hypertrophy response (Figure 1). Rabbits were divided into 3 groups of 10 rabbits according to the intervention: PVE alone, HVE alone, and a combination of HVE and PVE. All groups were subdivided into two subgroups (n=5 per subgroup), which were sacrificed 1 or 7 days after the intervention, respectively. These time intervals were chosen on the basis of previous studies, which showed that the hypertrophy response of the rabbit liver reaches a plateau phase 7 days after PVE. In the 7 days survival groups, CT volumetry was performed before embolization and 3 and 7 days after embolization for volumetric measurements and evaluation of revascularisation of the hepatic veins. Blood samples were drawn 3 hours and 1, 3, and 7 days after embolization. A portogram was performed before and directly after the intervention, as well as prior to sacrifice to confirm total occlusion of the portal vein. After sacrifice, liver tissue samples were excised and stored at -80°C or fixed in 4% formaldehyde.

**Interventions**

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 Corporation, Espoo, Finland). The eyes were protected from drying out using an eye cream (Oculentum simplex, Pharmachemie, Haarlem, the Netherlands). After subcutaneous injection of 0.03 mg/kg buprenorphine (Temgesic,
Reckitt Benckiser Healthcare, Hull, Great-Britain) and 0.2 mg/kg Baytril (Bayer Healthcare, Berlin, Germany), the rabbit was placed in supine position. Isoflurane 1-2% (Forene, Abbott Laboratories, Kent, UK) with $\text{O}_2$ : air (1:0.7 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) on the hind leg throughout the procedure. Rabbits were given analgesic care by subcutaneous administration of 0.02 mg/kg Baytril once a day for 3 days postoperatively. Portal and hepatic vein embolizations were performed by an interventional radiologist (KPvL) with over 10 years of experience.

**Hepatic vein embolization**

The right internal jugular vein was exposed via a small incision in the neck. The jugular vein was cannulated with an 18 G catheter (Hospira Venisystems, Lake Forest, IL). Under fluoroscopic guidance using a mobile C arm (Oldelft Benelux, Veenendaal, The Netherlands), a Renegade 3 Fr microcatheter (Boston Scientific, Place Natick, MA) with a Transend-ex 0.014-inch wire (Boston Scientific, Place Natick, MA) was introduced into the 18 G catheter and guided through the heart and the inferior caval vein to the hepatic veins of the cranial liver lobes. A venogram was made against the flow direction. Sequentially, the microcatheter was positioned in the right, middle, and left hepatic vein, which were then embolized with multiple 3-7-mm coils (Boston Scientific, Place Natick, MA). The embolization of the veins started in the periphery of the vein and was continued more centrally to completely occlude the vein, without losing coils into the right atrium and the lung. Then the catheter was removed and the cannulated jugular vein was closed with a ligature.

**Portal vein embolization**

PVE was performed as described previously prior to HVE in the combined group to avoid acute severe portal hypertension after HVE. Briefly, a branch of the superior mesenteric vein was cannulated with a venflon after a midline laparotomy. A microcatheter with a guidewire was subsequently introduced into the portal vein and a portogram was made to identify the individual portal vein branches. The microcatheter was positioned in the main portal branch supplying the cranial liver lobes after passing the portal branch to the caudal liver lobe. A mixture of contrast agent (Visipaque, GE Healthcare, Waukesha, WI) with 300-500-μm PVA particles (Cook, Bloomington, IN) was injected until flow ceased in the periphery. This procedure was followed by a more central positioning of 3 platinum coils close to the cranial portal main branch, without interfering with the caudal portal main branch. To confirm total occlusion of the cranial portal vein trunk, portography was repeated at the end of the procedure. Portography was concluded by ligation of the access branch of the superior mesenteric vein, after which the abdomen was closed.
CT volumetry
After induction anaesthesia, a multiphase CT scan was performed using a 64-slice CT scanner (Brilliance 64-channel, Philips Medical Systems, Eindhoven, The Netherlands). Rabbits were placed in supine position. After a blank series, a contrast-enhanced scan was performed 15 s (arterial phase), 30 s (portal phase), and 45 s (venous phase) after contrast agent injection (4 mL Visipaque), followed by 3 mL of sterile 0.9% NaCl solution. 3D-reconstructions of the liver were made using reconstructed 2-mm axial slices. The total liver and the caudal liver lobe were manually delineated and total liver volume (TLV) and caudal liver volume (CLV) were calculated by integrated software (MxView 3.52, Philips). CLV before HVE was expressed as percentage of TLV using the formula:

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

After HVE, %CLV was calculated using the formula:

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

The increase in CLV was calculated using the formula:

\[
\text{Increase CLV} = \left(\frac{{\text{CLV}}_{\text{post-embolization}}}{{\text{CLV}}_{\text{pre-embolization}}} - 1\right) \times 100\%
\]

The degree of hypertrophy = %\text{CLV}_{\text{post-embolization}} - %\text{CLV}_{\text{pre-embolization}}

Biochemical parameters
Whole blood was centrifuged at 3000g and platelet-poor plasma was isolated. Plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were assessed as well-established liver damage parameters by routine clinical chemistry. Plasma gamma glutamyl transpeptidase and alkaline phosphatase were measured to assess the extent of bile duct congestion. In addition, plasma albumin was used as indirect parameter of liver synthetic function, whereas plasma bilirubin was used as an indirect measure of hepatic uptake and excretory function.

Caudal liver-to-body weight index
After sacrifice the weight of the caudal liver lobe was measured using a precision scale (Sartorius, Göttingen, Germany). To obtain the caudal liver-to-body weight index, the liver weight was divided by the body weight.
**Wet-to-dry weight ratio**
Liver tissue samples of the caudal and left lateral liver lobe were weighed directly after sacrifice (wet weight), kept at 60°C for 4 weeks, and weighed again (dry weight).

The wet-to-dry weight ratio was calculated by
\[
\frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100\%.
\]

**Histology**
Liver tissue samples of the caudal and left lateral liver lobes were fixed in 4% formaldehyde, dehydrated in graded steps of ethanol and xylene, embedded in paraffin, and cut in 5 μm sections. Hematoxylin and eosin (H&E) staining was performed to evaluate the degree of necrosis, apoptosis, sinusoidal dilatation, and inflammation. Additionally, sections were immunostained with diaminobenzidine (DAB)-conjugated anti-Ki-67 antibodies (monoclonal mouse anti-rat Ki-67 antigen, clone MIB-5, Dako Cytomation, Glostrup, Denmark) according to the manufacturer’s instructions. The immunostained sections were counterstained with hematoxylin. Ki-67- and hematoxylin-positive cells were counted in 5 randomly selected fields of view per section (20× magnification) on microphotographs. The proliferation index was defined as the percentage of Ki-67-positive cells per total number of nuclei in the field of view.

**Statistical analysis**
Data were expressed as mean ± SD. Overall differences between the groups were analyzed with the Kruskal-Wallis test. If this test indicated a significant difference, 3 separate Mann-Whitney U tests were used for each comparison. To correct for multiple testing, a Bonferroni-Holm adjustment was made with an adjusted alpha of 0.05 denoting the level of significance.

**Results**

**Survival**
All rabbits survived the experiments without any clinical problems. No postoperative complications and no signs of illness were observed in the period after embolization. In one rabbit in the 1-day survival HVE group, a coil migrated via the heart into the left pulmonal artery directly after placement, however without any clinical consequences. A new coil was placed to occlude the hepatic vein.
**Degree of occlusion**

On portography, performed directly after PVE and before sacrifice, all rabbits that had undergone PVE or a combination of PVE and HVE showed complete occlusion of the portal vein, except for one rabbit in the combined group sacrificed on day 1, which showed persistent portal flow in part of the cranial liver lobe.

Occlusion of the hepatic vein was verified by CT. In most rabbits some small venous side branches were still patent, but in general, almost the complete venous outflow tract was adequately occluded. During sacrifice the position of the coils was checked in relation to the venous system. In all rabbits, the coils were found in the 3 main hepatic veins.

**Table 1. CT volumetry data**

<table>
<thead>
<tr>
<th></th>
<th>Before the intervention</th>
<th>Day 3</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVE CLV [%]</td>
<td>22.8±1.9</td>
<td>20.1±0.9</td>
<td>23.6±1.2</td>
</tr>
<tr>
<td>Increase of CLV [%]</td>
<td>N.A.</td>
<td>-10.2±5.5</td>
<td>5.6±7.0</td>
</tr>
<tr>
<td>Degree of hypertrophy [%]</td>
<td>N.A.</td>
<td>-2.7±1.2</td>
<td>0.79±1.5</td>
</tr>
<tr>
<td>PVE CLV [%]</td>
<td>22.4±0.5</td>
<td>29.8±0.8</td>
<td>40.1±1.1</td>
</tr>
<tr>
<td>Increase of CLV [%]</td>
<td>N.A.</td>
<td>33.6±14.5</td>
<td>79.8±8.4</td>
</tr>
<tr>
<td>Degree of hypertrophy [%]</td>
<td>N.A.</td>
<td>7.5±0.9</td>
<td>17.7±1.5</td>
</tr>
<tr>
<td>HVE+PVE CLV [%]</td>
<td>18.8±0.6</td>
<td>26.9±1.3</td>
<td>38.2±2.1</td>
</tr>
<tr>
<td>Increase of CLV [%]</td>
<td>N.A.</td>
<td>43.4±7.5</td>
<td>103.6±10.5</td>
</tr>
<tr>
<td>Degree of hypertrophy [%]</td>
<td>N.A.</td>
<td>8.1±1.3</td>
<td>19.4±2.0</td>
</tr>
</tbody>
</table>

**Liver regeneration response**

Table 1 shows the changes in caudal liver volume after each procedure. The CLV increased more in the PVE and the PVE/HVE group compared to the HVE group 3 and 7 days after the procedure (p<0.01). The caudal liver volumes of the combined group were slightly larger than the PVE group, but there were no significant differences in CLV increase or degree of hypertrophy between the PVE and the combined group at any time point (Figure 2).

The caudal liver-to-body weight index supports these data, since this index was significantly higher in the PVE and PVE/HVE group compared to the HVE group on day 7 (p<0.01). However, the index was also significantly higher in the combined PVE/HVE group compared to the PVE group (p=0.008). On day 1, no significant differences in liver-to-body weight index between the 3 groups were observed.

The liver volume/weight gain could have been caused by edema formation in the
caudal lobe. The wet-to-dry weight ratio is a parameter that represents the amount of fluid in the liver tissue samples. There were no significant differences in the wet-to-dry weight ratio between the 3 groups on day 1 and 7. Therefore the significant difference in body weight index cannot be explained by edema.
In accordance with the results above, the caudal liver of the PVE and combined group contained a significantly higher number of proliferating hepatocytes compared to the HVE group on day 7 (p<0.01) (Figure 3). The number of proliferating hepatocytes in the PVE/HVE group was not significantly higher than in the PVE group.

Liver damage and liver function
Plasma AST, ALT, and LDH plasma levels showed transient elevation in the first hours to day, without significant differences between the groups. All levels returned to baseline level on day 7 (Figure 4). No changes in plasma gamma glutamyl transpeptidas, alkaline phosphatase, and bilirubin were observed directly after the procedure or in the follow-up period (data not shown).

Histology
H&E slides obtained from the caudal and cranial left lateral liver lobes were evaluated by an experienced pathologist experienced in hepatic histopathology. A multinucleated giant cell reaction was seen around the embolization particles in the PVE animals as described before. There were no striking changes in liver architecture in the PVE group. In the cranial left lateral liver lobe, periportal and pericentral sinusoidal dilatation was observed in the PVE and combined group, while pericentral dilatation was seen particularly in the HVE group. Unexpectedly, marked changes were observed in the caudal liver lobe of the combined group. There was pericentral and periportal sinusoidal dilatation in the HVE group. However, in the combined
group, periportal sinusoidal dilatation in conjunction with atrophy of hepatocytes and local necrosis were clearly seen in all rabbits. Only little inflammation was observed. These observations apply for tissue samples obtained on days 1 and 7, although the necrosis was less pronounced in the former. One rabbit in the LVE group showed strong pericentral congestion and hepatocellular atrophy and necrosis in the lateral lobe on day 7, which could not be explained.

Discussion

In this experimental study, we assessed the value of HVE in addition to PVE for the increase of the FRL. The combined PVE and HVE was well tolerated by the animals. Clinically as biochemically, there were no signs of substantial liver damage, portal hypertension, or bile retention. Hepatic vein embolization in a rabbit is a challenging
The liver receives dual blood supply, i.e., from the portal vein as well as the hepatic artery. Supplying about 75% of the liver's blood pool, the portal vein carries venous blood drained from the splanchnic system. Besides PVE, HAE may be employed to reduce blood flow in a normal hepatic sinusoid. However, there are studies showing that this combined technique of PVE and HAE does not lead to an increased hypertrophy response. In this study, we showed that there is also no additional effect of HVE to PVE on the liver regeneration rate. Although these interventions were performed sequentially by Hwang et al., we think that the additional value of HVE is nihil. We have doubts concerning their conclusion that the additional liver regenerative response after HVE is explained by the HVE. HVE was performed 1-2 weeks following PVE, and the increase in FRL could still be the result of ongoing hypertrophy caused by PVE alone. Particularly, as we know that hypertrophy response continues for at least 3 weeks in humans, before a plateau phase is reached. The results of this study suggest that although histological and additional regenerative changes are seen, HVE in addition to PVE has no significant effect on the hypertrophy response. The combination of HVE and PVE may therefore, have little use in a clinical setting.
References


