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Short term effects of combined hepatic vein embolization and portal vein embolization for the induction of liver regeneration in a rabbit model

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Abstract

Background: Alternative methods to optimize the hypertrophy response after portal vein embolization (PVE) are desired. In this study we assessed the effect of hepatic vein embolization (HVE) in addition to PVE on the liver hypertrophy response in a standardized rabbit model.

Methods: Thirty rabbits were allocated to a group according to the intervention: PVE alone, HVE alone, and a combination of HVE and PVE. The liver regeneration response of the non-embolized, 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). 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: Although histological and additional regenerative changes are seen, HVE in addition to PVE, has no additional short term effect on the hypertrophy response. The combination of HVE and PVE may therefore, have little use in a clinical setting.
Introduction

Portal vein embolization (PVE) is now a widely used method to increase the future remnant liver (FRL) before major liver resection (1,2). PVE is considered when the FRL is considered too small, thereby increasing the risk of postoperative liver failure (3). With PVE, the portal vein branches of the liver lobe to-be-resected are occluded, causing atrophy of this liver lobe. Liver mediating cytokines, including TNF-α, interleukin IL-1β and IL-6, are released from Kupffer cells and activate hepatocytes to proliferate, inducing a compensatory hypertrophy response in the contralateral FRL (4-6). In most cases, the hypertrophy response following PVE reaches its plateau after 21 days(7). Thereafter, only little additional growth is seen . When the FRL volume, measured by CT volumetry 3 weeks after PVE, is ≥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 induction of tumor growth after PVE, potentially leading to secondary unresectability(8). Therefore, a technique should be developed that leads to a more rapid and reliable increase in FRL without tumour induction.

Recently, a Chinese research group published on sequential, ipsilateral hepatic vein embolization (HVE) after PVE in humans. In 12 patients who showed insufficient increase in volume of the future remnant liver after PVE, the right hepatic vein was also embolized to induce additional hypertrophy (9;10). The additional HVE was performed 13.5 ± 4.2 days after PVE. Embolizing the ipsilateral hepatic vein, theoretically should block the hepatic outflow and in combination with PVE, should decrease compensatory arterial hyperperfusion, thereby inducing additional hypertrophy of the non-embolized, future remnant liver. However, in this study, the increase in FRL could still be the result of ongoing hypertrophy caused by PVE alone. Nevertheless, 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.

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. 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.

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 2,470-3,430g) were acclimatized for 2 weeks under standardized laboratory conditions

Experimental design

A validated rabbit model was used for this study (11,12) The liver of the rabbit has four main lobes of which three are located cranially and one is located caudally. Only the three
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. (Figure1)

Rabbits were divided into 3 groups of intervention, comprising 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. Previous studies showed that the hypertrophy response of the rabbit liver reaches a plateau-phase 7 days after portal vein occlusion(11;12). Therefore we chose in this design only a 1 and 7 days survival group.

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). After subcutaneous injection of 0.03mg/ kg buprenorphine (Temgesic, Reckitt Benckiser Healthcare Limited, Hull, Great-Britain) and 0.2mg/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 (1:0.7L/min) was used to maintain anaesthesia. Rabbits were given the analgesic drug Baytril, 0.02mg/kg subcutaneously once a day for 3 days postoperatively. Portal and hepatic vein embolizations were performed by an interventional radiologist (KPvL) with over 10 years experience.

Hepatic Vein Embolization
The right jugular vein was cannulated with an 18 gauge 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 subsequently introduced into the 18G catheter and guided through the heart into the hepatic veins of the cranial liver lobes. A venogram was made against the flow direction. The microcatheter was sequentially 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 At the end of the procedure the catheter was removed and the cannulated jugular vein was closed with a ligature.
Portal Vein Embolization

PVE was performed prior to HVE in the combined group. The PVE-procedure was performed as described previously(11). Briefly, a branch of the superior mesenteric vein was cannulated with a venflon® after midline laparotomy. After introduction of a microcatheter in the portal vein, a portogram was made, visualizing the portal antomy. Then the microcatheter was positioned in the main portal branch supplying the cranial liver lobes. A mixture of contrast (Visipaque, GE Healthcare, Waukesha, WI) with 300-500 μm PVA particles (Cook, Bloomington, IN) was injected until flow ceased in the periphery, 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 trunc, 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 of anaesthesia, a multiphase CT scan was performed using a 64-slice CT scan (Brilliance 64-channel, Philips, Eindhoven, The Netherlands). Rabbits were placed in supine position. After a blank series, a contrast enhanced scan was performed 15 sec (arterial phase), 30 sec (portal phase) and 45 sec (venous phase) after contrast injection (4mL Visipaque, GE Healthcare, Waukesha, WI), followed by 3 ml 0.9% NaCl. 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 (Mx-View 3.52, Philips Medical Systems, Eindhoven, The Netherlands)

CLV before HVE was expressed as percentage of TLV using the formula:

\[
\%CLV_{\text{pre-embolization}} = \frac{CLV_{\text{pre-embolization}}}{TLV_{\text{pre-embolization}}} \times 100\%
\]

After HVE, %CLV was calculated using the formula:

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

Increase in CLV was calculated using the formula:

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

The degree of hypertrophy = %CLV_{\text{post-embolization}} - %CLV_{\text{pre-embolization}}
Biochemical parameters

In all blood samples 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 amount 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, this weight was divided by the body weight in order to exclude the influence of 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, embedded in paraffin, and cut in 5-μm sections. Hematoxylin-eosin (H&E) staining was performed to evaluate architectural changes. 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× magnifications) on microphotographs. The proliferation index was defined as the percentage of Ki-67-positive cells per total cells 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

No postoperative complications and no signs of sickness in the period after the embolization were observed.

In one rabbit of the 1day survival HVE group, a coil migrated during the procedure via the heart into the left pulmonary artery directly after placement, however without any clinical consequences. A new coil was placed that occluded the hepatic vein.

Degree of occlusion

On portography, performed directly after PVE and before sacrifice, all rabbits that underwent 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.

On CT scan, in most rabbits some small venous side branches were still patent, but in general, almost the complete venous outflow tract of the cranial lobe was adequately occluded. During sacrifice the position of the coils was checked in relation to the venous system to identify possibly missed and non-embolized venous branches. In all rabbits, the coils were found in the 3 main hepatic veins.

Liver regeneration response

Table 1 shows the changes in caudal liver volume after each procedure. The 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).

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 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). On day 1, no significant differences in body weight index between the 3 groups were present yet. Because the volume/weight gain could have been caused by edema formation in the caudal liver lobe, the wet-to-dry weight ratio was
assessed as a parameter representing 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 sacrificed on day 7, nor on day 1. Therefore edema does not account for the significant difference in body weight index. Also calculating the total body weight gain/loss of all rabbits during the experiment, no significant differences were seen between the groups (p=0.083).

In accordance with the results as mentioned above, the caudal liver of the PVE and combined group contained a significantly higher number of proliferating hepatocytes in the Ki-67 stained slides compared to the HVE group on day 7 (p<0.01). The number of proliferating hepatocytes of the PVE/HVE group was higher than the PVE group, only this difference was not significant (Figuur 3).
Liver damage

Biochemical results

Plasma AST, ALT, and LDH plasma levels showed transient elevation in the first hours to days, without significant differences between the groups. All levels returned to baseline levels 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 a pathologist experienced in liver pathology. Around the particles in the PVE groups, a multinucleated giant cell reaction was seen as described before(11). 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, whereas in the HVE group, pericentral dilatation was seen. Unexpectedly, substantial 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 the hepatocytes and local necrosis were clearly seen in all rabbits. Only little inflammation was observed. These observations apply for tissue samples obtained on day 7, just as on day 1, although necrosis was less pronounced in the latter.

**Discussion**

Besides PVE, there are other ways to reduce blood flow in a normal hepatic sinusoid. One of the strategies could be hepatic artery embolization (HAE). However, there are studies showing that the combination of PVE and HAE, compared to PVE alone, does not lead to an increased hypertrophy response(13). A recent study comparing HAE with PVE, also concluded that PVE was significant superior inducing hepatic hypertrophy (14).

In this experimental study, we assessed the value of HVE in addition to PVE. To eliminate the influence of the time factor after PVE and to achieve the maximum hypertrophy result in a short follow-up time, we performed PVE and HVE in one single procedure instead of sequentially. The results were satisfactory, as evidenced by the follow-up CT scans. Although some small patent venous side branches were seen, all three major hepatic veins of the cranial lobe were completely occluded in all rabbits. The effect of HVE alone on hypertrophy of the FRL was negligible. Although flow changes in the embolized lobes were present, and changes were seen on the histological slides, this did not result in increase of the FRL volume.

The combination of PVE/HVE and PVE alone resulted in a significantly greater hypertrophy response than HVE alone, as measured by CT volumetry, caudal liver to body weight index, and amount of proliferating hepatocytes.
The hypertrophy response after PVE/HVE was also significantly higher than after PVE alone as shown by the increased caudal liver to body weight ratio. The increase of CLV and the amount of proliferating hepatocytes showed the same trend, although the differences were not significant after 1 week.

The differences in liver volume and weight cannot be explained by edema formation, while no differences in wet-to-dry weight ratio were observed. Therefore, additional embolization of the hepatic veins after PVE had no significant additional effect on liver volume in the period of strongest hypertrophy response.

The histological changes and the significant increase in caudal liver-to-body weight ratio suggest that there is an additional effect on liver regeneration, only this effect did not translate into significant increase of volume of the FRL. There may be a long term effect, but this was beyond the scope of the present study as our interest focused on the short term effects of combined HVE/PVE embolization on the hypertrophy response.

As in most animal studies, one of the limitations is human extrapolation of the results. Although there are some differences, the rabbit liver shows great similarities in pathophysiological and anatomical aspect, demonstrated in previous publications (11). The regeneration response in the rabbit is faster, but the response curve is similar, therefore results can be extrapolated to the human situation (11,15)

Summarizing the results of this study, we suggest that although histological and additional regenerative changes are seen, HVE in addition to PVE has no additional short term effect on the volumetric hypertrophy response. The combination of HVE and PVE may therefore, have little use in a clinical setting.
References


