Increased Hepatic Microvascular Density, Oxygenation, and VEGF in the Hypertrophic Lobe following Portal Vein Embolization in Rabbits

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Keywords

Portal vein embolization · Liver microcirculation · Oxygenation · Liver regeneration · Liver atrophy and hypertrophy

Abstract

Introduction: The microvascular events following portal vein embolization (PVE) are poorly understood despite the pivotal role of the microcirculation in liver regeneration and tumor progression. We aimed to assess the changes in hepatic microvascular perfusion and neo-angiogenesis after experimental PVE. **Methods:** PVE of the cranial liver lobes was performed in 12 New Zealand White rabbits divided into 2 groups of permanent (P-PVE) and reversible PVE (R-PVE), respectively. Hepatobiliary scintigraphy and CT were used to evaluate hepatic function and volume. Hepatic microcirculation was assessed using a handheld vital microscope (Cyto-cam) to measure microvascular density (total vessel density; TVD) before PVE, right after PVE, and 20 min after PVE, as well

as at 14 days (D14 post-PVE) and 35 days (D35 post-PVE). Additionally, on D35, microvascular PO₂ and liver parenchymal VEGF were assessed. **Results:** Eleven rabbits were included after PVE (R-PVE, *n* = 5; P-PVE, *n* = 6). TVD in the nonembolized (hypertrophic) lobes was higher than in the embolized (atrophic) lobes of the P-PVE group at D35 post-PVE (36.7 ± 7.2 vs. 23.4 ± 4.9 mm/mm²; *p* < 0.05). In the R-PVE group, TVD in the nonembolized lobes was not increased at D35. Function and volume were increased in the nonembolized lobes of the P-PVE group compared to the embolized lobes, but not in the R-PVE group. Likewise, the microvascular PO₂ and VEGF staining rate were higher in the nonembolized lobes of the P-PVE group at D35 post-PVE. **Discussion/Conclusion:** Successful volumetric and functional hypertrophy of the nonembolized lobe was accompanied by microvascular alterations featuring increased neo-angiogenesis, microvascular density, and microvascular oxygen pressure following P-PVE.

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Introduction

Preoperative portal vein embolization (PVE) is used to induce hepatic hypertrophy with the aim of increasing future remnant liver volume and function, allowing more extended liver resections [1–3]. PVE is a technique by which the tumor-bearing side of the liver is deprived of portal flow through embolic occlusion of the ipsilateral portal vein branch. PVE induces hypertrophy of the non-embolized lobe and atrophy of the embolized lobe, which is known as the atrophy-hypertrophy complex [4].

PVE is a widely used procedure showing low morbidity and almost zero mortality [5, 6]. An important disadvantage of PVE is, however, the occurrence of tumor progression in the embolized lobe and especially in the non-embolized liver lobe in the waiting time after PVE [7–9]. Tumor progression (6–31%) is the major reason for non-resectability after PVE and it has major impact on patient survival [4, 10, 11].

It is well known that tumor growth depends on local microcirculatory perfusion and oxygenation within the tumor-bearing organ [12]. Therefore, to gain insight into the events following PVE, there is a need to assess hepatic microcirculation before, shortly after, and over a longer period of time after PVE, translatable to the clinical scenario. The rabbit model is unique in that it allows monitoring of microcirculatory changes in a separately regenerating, nonembolized lobe after PVE. Functional and volumetric enhancement of the nonembolized lobe in a rabbit model of PVE was found to be comparable with the hypertrophy response in humans after PVE [13, 14].

This study hypothesized that the targeted enhancement of volume and function of the nonembolized lobe resulting from portal venous deprivation of the embolized lobe depends on adequate induction of angiogenesis and increased microvascular perfusion and oxygenation in the nonembolized lobe. The aim of this study was, therefore, to assess, using a dedicated model in rabbits, the changes in hepatic microvascular perfusion, oxygenation, and microvascular architecture after PVE.

Methods

Animals

A total of 12 female New Zealand White rabbits with similar weight (mean $\pm$ SD: 3,088 $\pm$ 252 g) were obtained from Charles River (Saint-Germain Nuelles, France). This animal model has been applied in several studies conducted by our research group, always using female rabbits to keep the model reproducible [13, 15–20]. Before the start of the experiments, the rabbits were all ac-

climatized for 1 week. The animals were fed regular chow and given water ad libitum, and they were housed individually with a 12-h dark-light cycle. Reporting of the experiments was in accordance with Animal Research Reporting of in vivo Experiments (ARRIVE) guidelines.

Experimental Design

Previous studies have shown that the rabbit is a suitable animal for selective PVE, as the cranial lobes are anatomically separated from the caudal lobe and can be easily distinguished [13, 14, 21, 22]. The rabbits were divided into 2 groups ($n = 6$ rabbits per group) in which the cranial liver lobes underwent permanent (P-PVE) or reversible PVE (R-PVE). P-PVE ($n = 6$) was performed by selective injection of polyvinyl alcohol particles (300–500 μ m; Cook, Bloomington, IN, USA) followed by platinum fibered coils (5.0 and 4.0 mm; Boston Scientific, Natick, MA, USA) under radiographic control [13]. The P-PVE method and technique in rabbits have been used in previous studies from our department [13, 14, 21, 22]. R-PVE was performed using fibrin tissue glue containing a mix of fibrin glue and 3,000 kUnits/mL concentrations of aprotinin (Tissucol®P Combi-Set; Baxter AG, Vienna, Austria) [14]. The aprotinin concentration was reduced to 1,500 kUnits/mL and mixed with fibrin glue according to the manufacturer's protocol. The aprotinin concentration was reduced by 50% to ensure a short duration of portal venous blockage, with recanalization of the embolized portal vein branch within 3 weeks. Recanalization of the embolized portal vein branch was checked weekly after PVE with contrast-enhanced CT images.

Surgery and PVE

Anesthesia was induced by intramuscular injection of 25 mg/kg ketamine (Nimatek; Eurovet, Bladel, The Netherlands) and 0.2 mg/kg medetomidine (Dexdomitor®; Orion, Espoo, Finland). Anaesthesia was further maintained using 1–2% isoflurane (Forane®; Abbott Laboratories, Sittingbourne, UK) mixed with oxygen:air (0.5:1; 1.5 L/min). All of the animals received one subcutaneous injection of buprenorphine (0.03 mg/kg; Temgesic®; Reckitt Benckiser Healthcare, Hull, UK) for postoperative analgesia after the operations and Baytril® (0.2 mg/kg; BayerHealthcare, Berlin Germany) for antibiotic prophylaxis daily until the third postoperative day.

After completion of a midline laparotomy, a branch of the inferior mesenteric vein was cannulated with an 18-G catheter (Hospira Venisystem, Lake Forest, IL, USA). A Renegade 3-Fr microcatheter (Boston Scientific) with a Transend®-ex 0.36-mm $\times$ 182-cm guidewire (BostonScientific) was subsequently introduced into the portal vein. Visualization of the individual portal vein branches was carried out using a mobile C-arm Exposcop 8000 (Ziehm Imaging, Nurnberg, Germany). The microcatheter was selectively placed in the main portal branch to the cranial lobe of the liver. Thereafter, the embolization materials were infused through the catheter to achieve embolization of the portal branches of the cranial liver lobes. In the P-PVE group, the embolization material contained polyvinyl alcohol particles and coils. The animals in the R-PVE group were embolized using the mix of aprotinin (1,500 kunit/mL) and fibrin glue, as described above.

After the embolization procedure, portal occlusion was confirmed by digital subtraction portography. Subsequently, the catheter was extracted and a ligature was used to close the mesenteric

vein branch. The abdomen was closed in 2 layers using a running Vicryl® 4/0 suture (Ethicon, Johnson & Johnson, Somerville, NJ, USA) and interrupted Mersilene 3/0® U-sutures (Ethicon).

Microcirculatory Imaging

The hepatic microcirculation was assessed during laparotomy using a handheld vital microscope (CytoCam; Braedius Medical, Huizen, The Netherlands) [23]. This handheld video microscope is based on the incident dark-field imaging technique. Briefly, the incident dark-field imaging method uses green light that is produced from circumferential light-emitting diodes (LED) located on the tip of the device at the end of the light guide. LED produce green light, and when the device is located on the organ surface the green light is absorbed by the Hb of red blood cells. Thereby, the red blood cells appear as dark globules and enable visualization of the microcirculation.

The recordings are analyzed offline using AVA software (Automated Vascular Analysis, version 3.2, Microvision B.V., Amsterdam, The Netherlands) [24]. Total vessel density (TVD), perfused vessel density (PVD), and vessel surface (VS) area are measured. TVD is the total length of all of the microvascular vessels related to the surface area (mm/mm²). PVD is the total length of the vessels with at least a continuous flow in the microvessels (sluggish [very slow flow] or continuous flow [normal flow; the individual red blood cells are identifiable in the capillaries]) related to the surface area (mm/mm²).

Hepatic microcirculation was assessed in the P-PVE and R-PVE groups. The cranial and caudal lobes were measured on the day of the PVE, i.e., before embolization at D0 (pre-PVE), within 1 min after embolization (1 min post-PVE), and 20 min after embolization (20 min post-PVE). On days 14 and 35 post-PVE, the rabbits underwent relaparotomy, at which time the cranial and caudal lobes were reassessed. The rabbits were euthanized on day 35 post-PVE and the liver lobes were excised for assessment of VEGF.

Liver Microvascular Oxygen Pressure

The oxygen-quenched phosphorescent decay time of a systemically infused albumin-conjugated phosphorescent dye (palladium[II]-meso-tetra[4-carboxyphenyl]-porphyrin[Pd-porphyrin]; Pd-TCCP) was used to assess the microvascular oxygen tension in the liver on D35. The techniques used with this method have been published previously [25]. The LED-based phosphorimeter [26] was placed on the liver surface of the rabbits, i.e., on the caudal, hypertrophic lobe and on the atrophic, cranial lobe. On each lobe, microvascular PO₂ was measured in 6 different areas, and the mean of these 6 values was considered as microvascular PO₂ of the assessed lobe.

Quantification of Remnant Liver Regeneration and Function

The methods used to quantitatively assess liver regeneration have been described in detail previously [13, 14]. Briefly, multi-phase contrast-enhanced CT was performed using a multislice helical CT scanner (Philips Medical Systems, Eindhoven, The Netherlands) on postoperative days 0, 14, and 35 with injection of contrast solution (3 mL Visipaque™; GE Healthcare, Waukesha, WI, USA) in the lateral ear vein under anesthesia. Three-dimensional reconstructions of the liver were made by superimposing sequential 2-mm axial slices, and the volumes of the total liver and nonembolized caudal lobes were calculated using integrated soft-

ware (MX-View 3.52; Philips Medical Systems, Best, The Netherlands). This software measures the volumes of the lobes in addition to the total liver volume. The volumetric share of the nonembolized lobe (NELV%) was calculated using the following formula:

$$\text{NELV\%} = \frac{\text{NELV}}{\text{TLV}} \times 100\% .$$

The liver volume increase of the nonembolized liver lobe (NELV) was measured using the following formula:

$$\text{NELV increase\%} = \frac{\text{NELV day x} - \text{NELV baseline}}{\text{NELV baseline}} \times 100\%$$

Function of the embolized, cranial lobes and the nonembolized caudal lobes was measured using technetium-99m (^{99m}Tc)-mebrofenin hepatobiliary scintigraphy on day 1 before PVE and at 7, 14, 21, 28 and 35 days thereafter. Hepatobiliary scintigraphy was performed on a dual head gamma camera (Symbia T16; Siemens, Munich, Germany). The animals were placed in a supine position with the heart and liver in the field of view. Rabbits were injected with 50 mBq of ^{99m}Tc-mebrofenin (Bridatec; GE Healthcare, Little Chalfont, UK), and image acquisition was immediately started for 5 min at a frequency of 12 min⁻¹. Details of the data analysis have been described elsewhere [13, 14]. The function of the nonembolized caudal lobes was assessed to show the functional increase after PVE.

Biochemical Parameters

Plasma alanine aminotransferase (ALT), asparagin aminotransferase (AST), albumin, and hemoglobin (Hb) were measured before embolization on D0 (pre-PVE) and 14 and 35 days after PVE (D14 and D35 post-PVE). Biochemical parameters were analyzed using a Cobas®8000 modular analyzer.

VEGF Immunohistochemistry

Immunohistochemical staining of liver parenchyma for VEGF was performed using anti-VEGFA monoclonal antibody (ab1316; Abcam Company, Cambridge, MA, UK). VEGF staining was scored semiquantitatively by 2 blinded observers, using a score between 1 and 4 (1, no staining; 2, weak staining; 3, moderate staining; and 4, strong staining) [1]. The means of the results of the 2 observers were calculated to express VEGF intensity [27, 28].

Statistical Analysis

Statistical analysis was performed with GraphPad Prism version 7.0a (GraphPad Software, LJ, USA). The Shapiro-Wilk normality test was used to determine whether the data was normally distributed or not. A repeated measures 2-way analysis of variance (ANOVA; 2 factors: time as a related sample factor and group as an independent sample factor) with post hoc Sidak correction tests for multiple analyses were used to determine inter- and/or intra-group differences. Ordinary one-way ANOVA with a Tukey multiple comparison test was used to define intergroup differences. An unpaired *t* test was used for nonparametric data obtained from 2 groups. Data are expressed as means ± SD and differences were considered significant at *p* < 0.05.

Results

Body Weight and Liver Weight of Rabbits

Total body weights did not significantly change over time and were not different between groups. Total liver weights were not significantly different between the R-PVE and P-PVE groups on day 35 post-PVE (online

suppl. Table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000517025).

Liver Microcirculatory Alterations after PVE

Total Vessel Density

TVD was not significantly different in the embolized and nonembolized lobes at 1 min post-PVE and 20 min

Fig. 1. Microcirculatory density and oxygen pressure changes after PVE. **a** TVD. **b** PVD. **c** VS area after 14 and 35 days of PVE. **d** Liver microcirculatory oxygenation. Values are presented as means $\pm$ SD. * $p < 0.05$ pre-PVE vs. other time points. # $p < 0.05$, P-nonembolized vs. P-embolized lobes. \$ $p < 0.05$, P-embolized lobes on day 35 vs. R-embolized lobes on day 35. & $p < 0.05$, R-nonembolized vs. R-embolized lobes. P-embolized, P-PVE group; P-nonembolized, P-PVE group; R-embolized, R-PVE group; R-nonembolized, R-PVE group.

post-PVE both in the P-PVE group and in the R-PVE group (shown in Fig. 1a). TVD significantly changed after 14 and 35 days in the P-PVE group; TVD in the P-PVE group was significantly higher in the nonembolized lobes when compared to the embolized lobes on D14 (33.9 ± 6.9 vs. 23.9 ± 7.8 mm/mm², respectively; $p < 0.05$) and D35 (36.7 ± 7.2 vs. 23.4 ± 4.9 mm/mm², respectively; $p < 0.05$). These changes in TVD were confirmed in imaging studies of the hepatic microcirculation (Fig. 2). TVD in the embolized lobe of the P-PVE group showed a signifi-

cant decrease on D35 compared to baseline (32.1 ± 3 vs. 23.4 ± 4.9 mm/mm², respectively; $p < 0.05$), demonstrating a decrease in microvasculature in the atrophied, embolized lobes. On day 35 post-PVE, the TVD in the embolized lobes of the P-PVE group was significantly lower than in the embolized lobes of the R-PVE group (P-PVE: 23.4 ± 4.9 mm/mm² vs. R-PVE: 34.7 ± 3.8 mm/mm²; $p < 0.05$; Fig. 1a).

Perfused Vessel Density

The PVD in the P-PVE group was significantly higher in the nonembolized lobes compared to the embolized lobes on D35 (embolized: 23.4 ± 4.9 mm/mm² vs. nonembolized: 36.7 ± 7.2 mm/mm²; $p < 0.05$; Fig. 1b). The embolized lobe in the P-PVE group showed a significantly lower PVD on day 35 compared to baseline (day 35: 23.4 ± 4.9 mm/mm² vs. baseline: 32.1 ± 3 mm/mm² $p < 0.05$). In the R-PVE group, the PVD in the embolized and non-

Fig. 2. Screenshot images of CytoCam video measurements of the hepatic microcirculation at different time points before and after P-PVE in nonembolized (caudal) and embolized (cranial) lobes. The direct (1 and 20 min post-PVE) reaction in the nonembolized lobe represents vascular adaptation, larger diameters, and more filling of the vascular bed. The long-term response (on days 14 and 35 post-PVE) shows a new sinusoidal morphology, indicating microvascular remodeling in the nonembolized lobe and vascular deterioration in the embolized, atrophic lobe. Pre-PVE, the embolized and nonembolized (caudal) lobes show the same microvascular structure before embolization (sinusoidal microvasculature with varying diameters). One minute post-PVE, the embolized lobe shows smaller-diameter vessels as these are less filled with portal blood flow. The vessels in the nonembolized lobe show larger diameters and a higher density because of more portal filling with blood of the sinusoids. This effect of portal hyperperfusion after embolization represents vascular adaptation through an increased diameter of the sinusoids and a higher vessel density compared to baseline before embolization. Twenty minutes post-PVE, the embolized lobe shows a higher vessel density, whereas the nonembolized lobe shows almost the same morphology as at 1 min post-PVE. On day 14 post-PVE, the embolized and nonembolized lobes show signs of vascular remodeling. The embolized lobe shows a total loss of sinusoidal microvascular structure compared to the baseline pre-PVE value; the sinusoids do not connect with each other and show larger diameters of the sinusoids and white spots identified as collagen depositions from fibrosis. The nonembolized lobe shows a decrease in the diameter of the sinusoids, along with elongation of the sinusoidal structures. On day 35 post-PVE, the embolized lobe shows more disorganization of the sinusoidal microvasculature and larger white spots, indicating more collagen depositions. The nonembolized lobe shows smaller diameters and longer sinusoids. The vascular remodeling present on day 35 represents neo-angiogenesis in the nonembolized lobe that has occurred during hypertrophy, with a changed morphology compared to the pre-PVE, 1 min post-PVE, and 20 min post-PVE.

Fig. 3. Liver volume (a) and function (b) changes in nonembolized liver lobes after PVE. Values are presented as means $\pm$ SD. * $p < 0.05$, day 0 pre-PVE vs. day 14 post-PVE, day 21 post-PVE, day 28 post-PVE, and day 35 post-PVE. + $p < 0.05$, P-PVE group vs. R-PVE group. MUR, mebrofenin uptake rate.

Table 1. Biochemical parameters

Parameter	P-PVE			R-PVE		
	D0 pre-PVE	D14 post-PVE	D35 post-PVE	D0 pre-PVE	D14 post-PVE	D35 post-PVE
AST, units/L	34.8 $\pm$ 8	35.2 $\pm$ 15	32.4 $\pm$ 26	31.6 $\pm$ 11	32.2 $\pm$ 10	24.7 $\pm$ 7
ALT, units/L	41.8 $\pm$ 6	59.8 $\pm$ 15	61.2 $\pm$ 26	41.8 $\pm$ 10	48.6 $\pm$ 25	54 $\pm$ 23
Albumin, g/L	49.5 $\pm$ 5	47.6 $\pm$ 3	48.4 $\pm$ 3	52 $\pm$ 3	48.2 $\pm$ 2	50.8 $\pm$ 3
Hb, mmol/L	7.5 $\pm$ 1	7.5 $\pm$ 0	7.6 $\pm$ 0	7.6 $\pm$ 0	7.6 $\pm$ 0	7.6 $\pm$ 0

embolized lobes, however, did not change significantly over time. The PVD in the embolized lobes was significantly lower on day 35 post-PVE in the P-PVE group compared to the R-PVE group (23.4 ± 4.9 vs. 34.7 ± 3.8 mm/mm², respectively; $p < 0.05$; Fig. 1b).

VS Area

The VS in the nonembolized lobes increased at 1 min post-PVE in the P-PVE and R-PVE groups, but the values were only significantly increased at 20 min post-PVE compared to pre-PVE in both groups (in the P-PVE group, pre-PVE: $34.7 \pm 1.5\%$ vs. 20 min post PVE: $40.3 \pm 3.9\%$; $p < 0.05$; in the R-PVE group, pre-PVE: $34.6 \pm 2.9\%$ vs. 20 min post-PVE: $42 \pm 2.8\%$; $p < 0.05$; Fig. 1b). VS in the embolized lobes in both the P-PVE group and the R-PVE groups showed no significant differences at 1 min post-PVE and 20 min post-PVE (Fig. 1c). This finding is explained by the fact that, in the short term, the portal vein branches to the cranial lobes in both the R-PVE

group and the P-PVE group were initially blocked in the same way. However, in the R-PVE group over time, the embolization material was absorbed, resulting in less of a decrease in VS on D35 (P-PVE group: nonembolized vs. embolized lobes on D35 post-PVE: 39.8 ± 4.5 vs. $30.6 \pm 4.7\%$, respectively; $p < 0.05$; R-PVE group: nonembolized vs. embolized lobes on D35 post-PVE: 40.2 ± 2.2 vs. $35.2 \pm 7.6\%$, respectively; $p > 0.05$).

Liver Microvascular Oxygenation after PVE

The liver microvascular oxygen pressure on D35 was significantly higher in the nonembolized lobe compared to the embolized lobe in the P-PVE group (34 ± 12.7 vs. 65 ± 6.5 mm Hg, respectively; $p < 0.05$; Fig. 1d). No significant differences in liver microvascular oxygen pressure were found in the embolized and nonembolized lobes in the R-PVE group.

Liver Regeneration after PVE: Volume and Function of Nonembolized Liver Lobes

The results of CT volumetry and global liver uptake rates of ^{99m}Tc -mebrofenin scintigraphy are shown in Figure 3. The volume of the nonembolized lobes was significantly increased in the P-PVE group on days 14 and 35 post-PVE as compared to pre-PVE (day 14: $97.7 \pm 42.3\%$ and day 35: $190 \pm 98.2\%$ vs. pre-PVE baseline; $p < 0.05$), whereas in the R-PVE group the nonembolized lobes did not show any significant increase (Fig. 3a).

In the P-PVE group, the liver function of the nonembolized lobe was enhanced on day 7 but it did not further increase during the 35-day post-PVE period (day 0: $6.8 \pm 4.9\%$ min. vs. day 7: $34.2 \pm 9.7\%$ min.; $p < 0.05$). No increase in liver function was found in the nonembolized liver lobe of the R-PVE group. The nonembolized liver lobe function in the R-PVE group was significantly lower than in the P-PVE group on D14 (15.6 ± 10.5 vs. $35.4 \pm 10.4\%$ min.; $p < 0.05$, respectively), D21 (12.6 ± 10 vs. $38.9 \pm 7.6\%$ min.; $p < 0.05$, respectively), D28 (16.6 ± 11 vs. $36.4 \pm 8.5\%$ min.; $p < 0.05$, respectively) and D35 (14.3 ± 7.3 vs. $44.9 \pm 12.3\%$ min.; $p < 0.05$, respectively) post-PVE (Fig. 3b).

Biochemical Parameters

There were no significant differences between time points or groups in terms of AST, ALT, albumin, Hb values, and hemodynamic parameters (Table 1). Plasma ALT and AST values as indicators of liver damage were stable during the measurements at 14 and 35 days post-PVE in both the R-PVE group and the P-PVE group compared to baseline.

Neo-Angiogenesis: VEGF Intensity on D35

Only the nonembolized liver lobes in the P-PVE group showed a significantly higher VEGF compared to the embolized lobes (Fig. 4).

Discussion/Conclusion

In this study, we showed the alterations of hepatic microcirculation assessed by hand-held microscopy, liver tissue partial oxygen pressure, angiogenesis, and vessel architecture following PVE in a rabbit-model of selective PVE. Short and long-term effects were assessed using 2 different methods of PVE, i.e., P-PVE and R-PVE, with the latter providing a model of temporary portal vein occlusion. The main findings of this study are that the hypertrophy response after permanent embolization was

Fig. 4. VEGF liver parenchyma staining in the embolized and non-embolized lobes after 35 days of PVE. Quantification of immunohistochemical staining of VEGF in the embolized, atrophic and nonembolized, hypertrophic lobes of the liver 35 days after PVE in the P-PVE (a) and R-PVE (b) groups. * $p < 0.05$, embolized vs. nonembolized liver lobes in the P-PVE group. IHC, immunohistochemical.

associated with increased sinusoidal density, VS area, and microvascular oxygenation in the nonembolized lobe that had increased in volume and function. In addition, VEGF intensity levels were also higher in the nonembolized, hypertrophic lobes indicating induction of liver neo-angiogenesis and microvascular remodeling in comparison to the embolized, atrophic lobes. In contrast, reversible embolization did not result in a volume or functional increase in the absence of the microcirculatory features described with permanent embolization. The results of this study show that, with permanent embolization, prominent microcirculatory alterations and neo-angiogenesis (VEGF) are crucial factors associated with a successful volumetric and functional increase in the nonembolized lobe.

The high microvascular density (TVD and PVD) in the nonembolized hypertrophic lobes compared to the atrophic lobes described in this study is compatible with the findings of a clinical study in which the hepatic microcirculation was assessed intraoperatively after approximately 6 weeks of (permanent) PVE [29]. This study in patients undergoing liver resection also showed that the hypertrophic lobes revealed a higher microvascular density compared to the atrophic lobes. However, this clinical study lacked the baseline microvascular measurements because the percutaneous performance of PVE precluded open access to the liver. Also, no VEGF

or tissue microvascular oxygenation parameters were obtained in the clinical study. The present experiments exposed the full range of microvascular changes from baseline until 35 days after PVE, along with VEGF and microvascular oxygen pressure parameters. Herein, we demonstrated that redirection of the portal flow toward the nonembolized lobe increased angiogenesis, as assessed by a higher microvascular density and higher VEGF levels, to meet the high oxygen demand of the regenerating liver parenchyma. The hypertrophic lobes showed a higher microvascular oxygenation level compared to the atrophic lobe.

In the long term, the TVD was significantly higher in the hypertrophic nonembolized lobes at 14 and 35 days and the PVD was higher at 35 days when compared to the atrophic embolized lobes in the P-PVE group. These changes in TVD and PVD in the nonembolized lobes were accompanied by changes in the morphology of the vessels, indicating vascular remodeling. Long vascular structures represent elongated sinusoids with very small diameters compared to the baseline characteristics of the sinusoids before embolization. These findings are supported by the higher VEGF levels detected in the hypertrophic lobes of P-PVE rabbits, which are compatible with increased neo-angiogenesis during regeneration of the nonembolized lobes [15]. These findings suggest that 2 phases of microvascular changes occur during the hypertrophic phase, i.e., an immediate phase with microvascular (tone) adaptation and a delayed phase characterized by microvascular remodeling resulting from neo-angiogenesis.

In atrophic embolized lobes after P-PVE, we showed that TVD and PVD were depleted at 35 days in comparison to baseline, indicating an atrophic response of liver sinusoidal microcirculation. Gock et al. [30] showed that microcirculation of atrophic lobes was deteriorated with the reduction of sinusoidal perfusion, an elevation of sinusoidal diameter (increase in VS%), and cell apoptosis on days 1–3 after portal branch ligation. However, in the present study, the VS% of atrophic embolized lobes was not considerably changed until day 35 because of the arterial buffer response and late microvascular remodeling. Moreover, in parallel to our results, Kollmar et al. [31] demonstrated that the late microvascular remodeling in atrophic lobe was associated with normalization of blood flow, the arterial buffer response, sinusoidal dilatation, and arterIALIZATION 14 days after portal branch ligation in mice. Of note, the alteration of blood flow or perfusion can change the VS% but not the total length of the sinusoidal vessel (TVD). In our study, we also observed a re-

duction of the VS area throughout the experiment, but it did not reach to significance in the embolized lobes.

Enhanced angiogenesis is at the same time a concern in the setting of clinical PVE in patients with malignant liver tumors, as the newly formed vessels will promote tumor growth [16, 17]. Blocking neo-angiogenesis during liver regeneration after PVE using anti-VEGF medication (e.g., bevacuzimab) is a potential therapeutic intervention to avoid progression of the tumor in the interval between PVE and resection. However, anti-VEGF therapy may impair the liver-regenerative response, rendering VPE less effective. To date, findings on the use of anti-VEGF therapy after PVE are controversial, underscoring the need for further research [18–20].

R-PVE did not result in substantial hypertrophy of the nonembolized lobes, while no increase of volume or function was achieved. The short duration of portal venous occlusion resulted in an incomplete regeneration process. The embolized liver lobes in the R-PVE group were recanalized approximately 3 weeks after embolization, while at 14 days the portal venous system was still occluded. Microvascular assessment at 14 days showed an increase in TVD of the nonembolized lobe comparable to that in the permanently embolized group. This finding is not surprising as in the first 2 weeks after embolization the reversible embolization material was not yet absorbed and the portal vein was still occluded. After 3 weeks, the reversible embolization material had dissolved, which was the reason the embolized lobes did not show the same decrease in TVD as in the P-PVE group, in which a marked decrease in TVD was noted. This finding is essential in the context of the concept that the level of atrophy in the embolized lobe determines the extent of hypertrophy of the nonembolized lobe as the main driver of the regenerative process. Hence, regarding the atrophy-hypertrophy complex, we demonstrated that an adequate increase in the function and volume of the nonembolized lobes largely depends on depletion of sinusoidal microcirculation in the embolized lobes.

In conclusion, we showed in this rabbit model of PVE that microvascular alterations are associated with a successful atrophy-hypertrophy complex resulting from portal venous occlusion. Successful hypertrophy of the nonembolized lobe leading to increased volume and function was characterized by an increase in neo-angiogenesis, microvascular density, and microvascular oxygen pressure. Lastly, we suggest that P-PVE can provide sufficient remnant liver volume and function in parallel to the improvement of liver microcirculation, remodeling, and oxygenation.

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Statement of Ethics

The experimental procedures were approved by the institutional animal ethics and welfare committee of the Academic Medical Center (BEX35). Reporting of the experiments was in accordance with the Animal Research Reporting of in vivo Experiments (ARRIVE) guidelines.

Conflict of Interest Statement

C.I. has developed SDF imaging and is listed as an inventor on related patents commercialized by MicroVision Medical (MVM) under a license from the Academic Medical Centre (AMC). C.I. receives no royalties or any benefits from this license. He has been a consultant for MVM in the past but has not been involved with this company for more than 7 years and holds no shares or stock. Braedius Medical, a company owned by a relative of C.I., has developed and designed a third-generation handheld microscope called CytoCam-IDF imaging. C.I. has no financial relation with Braedius Medical of any sort, and has never owned shares or received consultancy or speaker fees from Braedius Medical. C.I. runs an internet site (<https://www.microcirculationacademy.org>) which offers services (e.g., training, courses, and analysis) related to clinical microcirculation. The other authors declare that they have no competing interests.

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Author Contributions

Z.U. drew up the study design, coordinated the experiment and schedule, assessed the hepatic microcirculation and perfusion in all samples, performed the analysis of all samples, interpreted the data, and drafted, wrote, and revised this paper. B.E. performed the analysis of all samples, interpreted the data, drafted and revised this paper, and gave approval for the version to be submitted. L.S. contributed to the microcirculatory data analyses and to data interpretation, revised this paper, and gave approval for the version to be submitted. K.P.L. helped to design and schedule the experiment, applied PVE in the rabbits, interpreted the data, draft and revised this paper, and gave approval for the version to be submitted. F.R. assessed the liver volume data, contributed to data interpretation, revised this paper, and gave approval for the version to be submitted. P.B.O. helped to design and plan the experiment, interpreted the data, revised this paper, and gave approval for the version to be submitted. R.J.B. provided the assessment of liver volume and liver function, interpreted the data, drafted and revised this paper, and gave approval for the version to be submitted. C.I. contributed to the microvascular perfusion assessment of this experiment, provided the training, software, and devices to assess microcirculation and perfusion, interpreted the data, draft and revised this paper, and gave approval for the version to be submitted. T.M.G. had the initial idea for this experiment in the well-established rabbit model of his experimental surgery laboratory, drew up the study design, provided the laboratory tools, guided the researchers involved in this experiment, interpreted the data, draft and revised this paper, and gave approval for the version to be submitted.

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