Portal branch ligation induces a hepatic arterial buffer response, microvascular remodeling, normoxygenation, and cell proliferation in portal blood-deprived liver tissue

Otto Kollmar,1 Marcus Corsten,2 Claudia Scheuer,2 Brigitte Vollmar,3 Martin K. Schilling,1 and Michael D. Menger2

1Department of General, Visceral, Vascular, and Pediatric Surgery and 2Institute for Clinical and Experimental Surgery, University of Saarland, Homburg-Saar; and 3Department of Experimental Surgery, University of Rostock, Rostock, Germany

Submitted 28 October 2006; accepted in final form 5 March 2007

Portal branch ligation induces a hepatic arterial buffer response, microvascular remodeling, normoxygenation, and cell proliferation in portal blood-deprived liver tissue. Am J Physiol Gastrointest Liver Physiol 292: G1534–G1542, 2007. First published March 8, 2007; doi:10.1152/ajpgi.00503.2006.—Portal branch ligation (PBL) may prevent liver failure after extended hepatic resection. However, clinical studies indicate that tumors within the ligated lobe develop accelerated growth. Although it is well known that tumor growth depends on the host’s microvascularization, there is no information about how PBL affects the hepatic microcirculation. Our aims were to determine hepatic artery response, liver microcirculation, tissue oxygenation, and cell proliferation after PBL. Therefore, we used intravital multilocus microscopy, laser-Doppler flowmetry, immunohistochemistry, and biochemical techniques to examine microcirculatory responses, microvascular remodeling, and cellular consequences after left lateral PBL in BALB/c mice. During the first 7 days, PBL induced a reduction of left hilar blood flow by ~50%. This resulted in 80% sinusoidal perfusion failure, significant parenchymal hypoxia, and liver atrophy. After 14 days, however, left hilar blood flow was found to be restored. However, remodeling of the microvasculature included a rarefaction of the sinusoidal network, however, without substantial perfusion failure, compensated by a hepatic arterial buffer response and significant sinusoidal dilatation. This resulted in normalization of tissue oxygenation, indicating arterialization of the ligated lobe. Interestingly, late microvascular remodeling was associated with increased endothelial nitric oxide synthase expression, significant hepatocellular proliferation, and weight gain of the ligated lobe. Thus PBL induces only an initial microcirculatory failure with liver atrophy, followed by a hepatic arterial buffer response, microvascular remodeling, normoxygenation, and hepatocellular proliferation. This may explain the accelerated tumor progression occasionally observed in patients after PBL.

LIVER RESECTION IS CONSIDERED to be the best treatment option for patients with malignant liver tumors and metastases. The loss of functional liver mass after hepatectomy is compensated by the regenerative capacity of the liver to regain the initial volume and function. Extensive hepatic resections beyond 75% of the liver mass, however, are associated with an inadequate regenerative reserve of the remaining parenchyma. This results in parenchymal dysfunction and liver failure, which is associated with a high mortality rate (17).

To reduce the risk of liver failure and to increase the number of resectable patients requiring extensive hepatectomy, portal vein occlusion has been introduced (11). Four to eight weeks before the planned hepatectomy, the individual portal vein branches that supply the tumor-bearing liver lobe are occluded by ligation (3) or embolization (1). This procedure has been demonstrated to induce atrophy of the portal blood-deprived lobe, whereas the nonligated contralateral tissue, which represents the remnant liver after later hepatectomy, undergoes compensatory hypertrophy (11).

Previous studies have shown that the hypertrophy of the nonligated lobe after portal branch occlusion develops a sufficient regenerative capacity for later hepatectomy (1, 7). However, little is known about whether the portal branch ligation (PBL)-induced atrophy of the tumor-bearing lobe affects nutritional blood supply and thus tumor growth. In contrast to the compensatory hypertrophy, which is initiated directly by an early proliferative response (9), the rapid shrinkage of the ligated lobe is thought to be associated with a reduction in size and number of hepatocytes, most probably due to sustained hypoxia and metabolic deprivation of portal hepatotrophic factors (15, 20, 34). The PBL-induced atrophy involves both centrolobular necrosis and apoptotic cell death in a time-dependent manner, however, without affecting the hepatic microarchitecture (15, 16, 20, 25, 29).

Interestingly, there is some indication from clinical studies that, despite liver atrophy, tumors within the ligated lobe do not shrink in size but rather show acceleration of growth (3, 7). In addition, experimental studies have indicated that the loss of portal blood flow after PBL is compensated by a hepatic arterial buffer response (HABR), which guarantees a rapid normalization of overall blood flow in the ligated lobe (22, 29).

Whether this arterialization affects the hepatic microcirculation has not been determined yet. This information, however, is of major importance, because growth and spreading of tumor tissue is known to essentially depend on the host’s overall nutritional microvascular blood supply. In the present study, we therefore analyzed the microcirculatory response and microvascular remodeling as well as their consequences on hepatic parenchymal cell proliferation and apoptosis after left PBL.

Address for reprint requests and other correspondence: O. Kollmar, Dept. of General, Visceral, Vascular, and Pediatric Surgery, Univ. of Saarland, D-66421 Homburg/Saar, Germany (e-mail: otto.kollmar@uniklinikum-saarland.de).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
MATERIALS AND METHODS

Animals and operative procedure. Experiments were performed after approval by the local governmental ethics committee and were in accordance with the U.S. National Research Council’s Guide for Care and Use of Laboratory Animals (http://books.nap.edu/readingroom/books/labrats/). Female BALB/c mice (18–20 g body wt) were used. Anesthesia was performed by intraperitoneal injection of 20 mg/kg body wt xylazine (Rompun; Bayer, Leverkusen, Germany) and 75 mg/kg body wt ketamine (Ketavet; Pharmacia, Erlangen, Germany). The animals were placed in a supine position. After laparotomy through a midline incision, the left lateral and medial liver lobes were gently mobilized. For PBL, the left portal vein feeding the left lateral liver lobe was exposed and ligated with a 9-0 suture (Ethilon, 0.3 metric BV-4; Ethicon, Norderstedt, Germany) using the finest microinstruments and stereomicroscopy. During preparation, meticulous care was taken that arterial blood vessels and bile ducts were not injured by the surgical procedure. To avoid intrathepatic portal venous blood flow through shunts from the left medial liver lobe, the portal vein of the left medial liver lobe was also exposed and ligated with 9-0 Ethilon. In sham-operated animals (controls), the corresponding portal veins were exposed but were not ligated. All liver lobes were repositioned anatomically into the peritoneal cavity. The abdominal wall was closed in a one-layer technique with a polypropylene suture (5-0 Prolene; Ethicon). After the operation, the animals were housed in single cages and were allowed free access to water and standard pellet food.

Experimental protocol. Thirty-two animals underwent PBL and were assigned to four different groups. All animals were examined by intravital microscopy, and the liver was harvested for histology and immunohistochemistry. Sham-operated animals served as controls (n = 4). PBL animals without long-term follow-up (n = 8) underwent intravital fluorescence microscopy before (baseline) as well as 30 and 60 min after PBL. The three groups of animals with long-term follow-up (n = 8 each) were examined on postoperative days 3, 7, and 14, respectively.

Intravital fluorescence microscopy. For intravital microscopy, the left lateral liver lobe was exteriorized and placed on an adjustable stage. The surface of the lobe was covered by a coverslip to avoid drying of the tissue and influences of the ambient oxygen (8). Microscopy was performed with an epi-illumination technique by using a modified Zeiss Axioscope microscope (Zeiss, Oberkochen, Germany) (12). Images were monitored by a charge-coupled device camera (FK-6990; Prospective Measurements, San Diego, CA) and were transferred to a video system for offline analysis. With the use of water-immersion objectives (×10/0.30; ×20/0.50 W; ×40/0.75 W), magnifications of ×150, ×300, and ×600 were achieved on the video screen (PVM-202M2E; Sony, Tokyo, Japan).

Angioarchitecture and microvascular perfusion were analyzed after intravenous contrast enhancement with sodium fluorescein (2 μmol/kg; Merck, Darmstadt, Germany) by using blue-light epi-illumination (450–490 nm excitation/>520 nm emission). Leukocyte adhesion in liver sinusoids was assessed after in vivo white blood cell staining with rhodamin-6G (2 μmol/kg; Merck) by using green-light epi-illumination (550–560 nm excitation/>580 nm emission). Parenchymal cells were visualized by in vivo nuclear staining with bisbenzimidazole (H-33342; 2 μmol/kg; Sigma-Aldrich) by using a near-ultraviolet filter system (330–380 nm excitation/415 nm emission) (23). Analyses were performed in 10 microscopic fields in each animal. In an additional five microscopic fields, hepaticellar NADH fluorescence was measured as an indicator of mitochondrial redox state (tissue oxygenation) (30).

Microcirculation analysis. Microcirculatory parameters were examined offline by using a computer-assisted image analysis system (CapImage; Zeintl, Heidelberg, Germany). Data analysis was performed by examiners unaware of the treatment. Sinusoidal density (per millimeter) was determined within the midzonal region of the liver acinus as total number of visible sinusoids crossing a 500-μm raster line (31). The functional sinusoidal density (per millimeter) was determined as the number of perfused sinusoids crossing the 500-μm raster line. The ratio of perfused sinusoids represents the percentage of perfused sinusoids in relation to all sinusoids visible. Sinusoidal diameters (in micrometers) were measured perpendicularly to the vessel path. The number of leukocytes that adhered to the sinusoidal lining for >20 s was counted as cells per microscopic field (per square millimeter) (23). The number of apoptotic cells was determined by counting the cells with condensation of bisbenzimid-stained nuclei per 1 mm² microscopic field (32). Hepatocellular NADH fluorescence was assessed densitometrically by computer-assisted gray-level determination (CapImage) (30).

Laser-Doppler flowmetry. Monitoring of left hilar blood flow, which feeds the left medial and lateral liver lobes, was performed by using a laser-Doppler flowmeter (floLAB; Moor Instruments, Millwley, UK) (4). Blood flow was continuously recorded for 20 s directly before as well as at days 3, 7, and 14 after PBL (arbitrary units, AU). Data are given as percentage of the initial blood flow values before PBL.

To directly document the HABR after PBL, we examined additional animals (n = 4) with a differential analysis of the left hepatic arterial flow and the left portal venous flow.

Laboratory parameters. Blood samples were taken at the end of the experiment. Activities of aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase were assessed by using standard enzymatic methods.

Histology and immunohistochemistry. At the end of the experiment, the weight of the whole liver and the left lateral lobe was determined. Formalin-fixed, paraffin-embedded tissue was stained with hematoxylin and eosin for routine histology. The score applied for semiquantitative analysis of intralobular necrosis, cytoplasmic clot formation, and vacuolation was 4 (>50%), 3 (31–50%), 2 (11–30%), 1 (1–10%), and 0 (0%).

Immunohistochemistry of PCNA was used to indicate proliferative activity. A monoclonal mouse-anti-pan-PCNA antibody (PC10, 1:50; DakoCytomation, Hamburg, Germany) was used as primary antibody, followed by a biotinylated goat-anti-mouse/rabbit-IgG antibody injection for streptavidin-biotin-complex peroxidase staining (Universal LSAB 2 Kit/HRP, 1:200; DakoCytomation). 3,3′-Diaminobenzidin (DakoCytomation) was used as a chromogen. Sections were counterstained with hemalun. Positively stained hepatocytes were scored semiquantitatively as 4 (>50%), 3 (31–50%), 2 (11–30%), 1 (1–10%), and 0 (0%).

Additional slices were exposed to an IgG isotype-matched antibody instead of the primary antibody. All of these control stainings were found negative.

Western blot analysis. For whole protein extracts and Western blot analysis of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS), liver tissue was homogenized in lysis buffer (10 mM Tris, pH 7.5, 10 mM NaCl, 0.1 mM EDTA, 0.5% Triton X-100, 0.02 mM NaN₃, 0.2 mM PMSF, and protease inhibitor cocktail 1:100 vol/vol; Sigma, Taufkirchen, Germany), incubated for 30 min on ice, and centrifuged for 30 min at 16,000 g. The supernatant was saved as whole protein extract fraction. Protein concentrations were determined by using the Lowry assay with bovine serum albumin as standard. Fifteen micrograms of protein per lane were separated discontinuously on 10% SDS-PAGE gels and were transferred to polyvinylidene difluoride membranes (0.2 μm; Bio-Rad, Munich, Germany). After blockade of nonspecific binding sites, membranes were incubated for 2 h with the rabbit polyclonal anti-mouse eNOS antibody (1:500; BD Transduction Laboratories, Heidelberg, Germany) and the rabbit polyclonal anti-mouse iNOS antibody (1:100; Santa Cruz Biotechnology, Heidelberg, Germany), followed by the secondary peroxidase-conjugated donkey anti-rabbit IgG antibody (1.5 h, 1:5,000; Amersham Pharmacia Biotech, Freiburg, Germany). Protein expression was visualized by means of luminol enhanced
chemiluminescence (ECL; Amersham) and exposure of the membranes to a blue light-sensitive autoradiography film (Hyperfilm ECL; Amersham). Signals were densitometrically assessed (Gel Doc, Quantity One software; Bio-Rad) and were normalized to β-actin signals (mouse monoclonal anti-β-actin; Sigma) to correct for unequal loading.

Statistical analysis. Data are given as means ± SE. After proving the assumption of normality and homogeneity of variance across groups, differences between groups were calculated by ANOVA followed by Dunnett’s or Dunn’s post hoc test. For repeated measurements, differences were calculated by repeated-measures ANOVA followed by Dunnett’s post hoc test. Overall statistical significance was set at P < 0.05.

RESULTS

All animals had an uneventful postoperative recovery. At day 3 after PBL, animals showed a significantly reduced body weight (16.6 ± 0.6 g; P < 0.05) compared with preoperative values (19.1 ± 0.5 g) and sham-operated controls (19.5 ± 0.5 g). Interestingly, mean body weight of the animals recovered to 20.1 ± 0.8 g and 19.3 ± 0.4 g at days 7 and 14, which was not different from that measured before PBL (20.1 ± 0.6 g and 19.9 ± 0.6 g).

Analysis of left hilar blood flow by laser-Doppler flowmetry revealed that PBL provokes a significant reduction to ~50% at day 3 compared with preoperative baseline. At day 14, however, perfusion of the left hilus almost completely normalized, with flow values (86.6 ± 14.7%) not significantly different from that measured before PBL and in sham-operated controls (Fig. 1A).

The whole liver weight increased slightly after PBL, with a significant peak at day 7 (1.01 ± 0.07 g) compared with sham-operated controls (0.86 ± 0.02 g; P < 0.05). In parallel to the reduction of left hilar blood flow, the left lateral liver lobe atrophied initially after PBL, with lowest values (15.9 ± 1.9% of whole liver weight) at day 7, but showed recovery with weight gain (22.0 ± 4.0%) at day 14 (Fig. 1B).

At baseline, intravital microscopy showed a regularly arranged hepatic microvascular architecture with almost 100% sinusoidal perfusion. PBL provoked a significant shutdown of 80% of the perfused sinusoids within the first 30 min, including both perfusion failure of individual sinusoids and complete sinusoidal networks (Figs. 2 and 3). This reduced functional sinusoidal density did not recover to normal throughout the entire 14-day observation period (Fig. 2C). Detailed analysis, however, showed that remodeling of the left lobe was associated with an overall rarefaction of the absolute number of sinusoids (Fig. 3C) but that the ratio of perfused sinusoids approached ~80% (Fig. 3D).
The initial deterioration of sinusoidal perfusion after PBL was associated with an accumulation of sodium fluorescein within the periportal regions of the acini (Fig. 2B), indicating a reduced hepatocellular clearance function. Interestingly, this accumulation of sodium fluorescein could no longer be detected during the later 14-day observation period (Fig. 3B).

Because angiogenesis is regularly associated with vasodilation, we analyzed sinusoidal diameters before and after PBL.

During the first 60 min after PBL, sinusoidal diameters were not found to be affected. During the later time course, PBL induced significant (P < 0.05) sinusoidal dilation, which was most pronounced at day 14 (Fig. 4).

Analysis of NADH fluorescence demonstrated a significant increase within the first 60 min after PBL, indicating impairment of tissue oxygenation (Fig. 5). Interestingly, from day 3

During the first 60 min after PBL, sinusoidal diameters were not found to be affected. During the later time course, PBL induced significant (P < 0.05) sinusoidal dilation, which was most pronounced at day 14 (Fig. 4).

Analysis of NADH fluorescence demonstrated a significant increase within the first 60 min after PBL, indicating impairment of tissue oxygenation (Fig. 5). Interestingly, from day 3...
to day 14 NADH fluorescence was found to be not only recovered but even below baseline values, indicating supranormal tissue oxygenation during the later time course after PBL.

To study whether this is due to a HABR, we performed differentiated laser-Doppler flowmetry analysis of the left hepatic arterial and the left portal venous blood flow. These experiments demonstrated that, under baseline conditions, portal venous flow and hepatic arterial flow comprise 447.7 ± 13.9 AU and 200.8 ± 11.1 AU (Fig. 6). At 30 min after PBL, flow in the portal vein was zero, whereas hepatic arterial flow was found significantly increased. After 14 days, a further increase of the hepatic arterial flow to 581.3 ± 11.6 AU, which represents a value almost as high as the overall hepatic arterial and portal venous flow during baseline, clearly indicated a HABR.

Hematoxylin and eosin-stained tissue sections revealed an increased frequency of cytoplasmic clot formation and vacuolization of parenchymal cells, which was most pronounced at day 14 after PBL (Fig. 7). Interestingly, these hepatocellular alterations were not restricted to the pericentral tissue regions but were found homogeneously distributed within the whole acini.

The intravital microscopic analysis of leukocyte adherence to the sinusoidal lining already showed a significant increase in the number of adherent leukocytes after PBL. Magnification, ×80. C: quantitative analysis of leukocyte adherence, measured as the number of cells per 1 mm² microscopic field before (baseline) as well as 30 min, 60 min, 3 days, 7 days, and 14 days after PBL. PBL induced significant leukocyte adherence within the first 60 min, which, however, was found to be markedly aggravated at day 3 and still significantly elevated at day 14. Values are means ± SE; *P < 0.05 vs. baseline.

Fig. 6. Left portal venous (A) and left hepatic arterial blood flow (B), as measured by laser-Doppler flowmetry, at baseline as well as 30 min and 14 days after PBL. Values are means ± SE; *P < 0.05 vs. baseline; #P < 0.05 vs. 30 min.

Fig. 7. Histological analyses (A and B) and semiquantitative scoring (C) of cytoplasmic clot formation in hepatocytes of the left lateral liver lobe (hematoxylin-eosin staining). Whereas liver tissue of sham-operated controls did not show any signs of cytoplasmic clot formation and vacuolization (A and C), liver tissue after PBL displayed an increasing amount of those intracellular alterations until day 14 (B and C). Score: cells with cytoplasmic clot formation and vacuolization >50% (4 points), 30–50% (3 points), 10–30% (2 points), 0–10% (1 point), and 0% (0 points). Values are means ± SE; *P < 0.05 vs. control. Magnification, ×175.

Fig. 8. Intravital microscopic analysis of sinusoidal leukocyte adherence at baseline (A) and 3 days after PBL (B). Note the massive increase of the number of adherent leukocytes after PBL. Magnification, ×80. C: quantitative analysis of leukocyte adherence, measured as the number of cells per 1 mm² microscopic field before (baseline) as well as 30 min, 60 min, 3 days, 7 days, and 14 days after PBL. PBL induced significant leukocyte adherence within the first 60 min, which, however, was found to be markedly aggravated at day 3 and still significantly elevated at day 14. Values are means ± SE; *P < 0.05 vs. baseline.
and lactate dehydrogenase increases had a documented delay until day 7 (data not shown).

Quantitative analysis of nuclear condensation demonstrated that PBL induces significant hepatocellular apoptosis. In contrast to necrotic injury, apoptotic cell death was found to be most pronounced at days 7 and 14 after PBL (Fig. 10).

Finally, analysis of cell proliferation revealed only a minor response during the first 7 days after PBL. However, at day 14 there was a pronounced increase in PCNA-stained nuclei, indicating a delayed proliferative response upon PBL (Fig. 11).

To study the role of nitric oxide on microcirculatory remodeling after PBL, eNOS and iNOS expression were analyzed by Western blotting. These analyses indicated a weak expression of eNOS at day 0 and a slight increase over the first 7 days after PBL. At day 14, however, eNOS expression was significantly increased to values that were threefold higher than that measured at day 0 (Fig. 12). Analysis of iNOS expression revealed a slight but not significant decrease at days 3 and 7 after PBL and values at day 14 that were comparable with that measured at day 0 (Fig. 12).

DISCUSSION

The major findings of the present study are that PBL induces only initial microcirculatory failure with atrophy of the deprived liver. This is followed by 1) restoration of hilar portal blood flow through a HABR, 2) microvascular remodeling, 3) parenchymal normoxygenation, and 4) hepatocellular proliferation.

Previous studies clearly indicate that occlusion of the portal vein induces a HABR to maintain total liver blood flow (10, 21). In the present study, we could demonstrate that left PBL provoked a 50% reduction of the left hilar blood flow at day 3, which, however, recovered to almost normal after 14 days. Assuming a 20–30% contribution of the hepatic artery to the total blood flow of normal livers (5), the 50% reduction of the left hilar blood flow at day 3 after PBL indicates a HABR-associated twofold increase of arterial flow. Because the mass of the left lobe had decreased to ~60%, this represents a
3.3-fold increase of arterial flow. Interestingly, at day 14 after PBL the overall left hilar blood flow had recovered to \( \frac{90}{100} \) of controls, representing a fourfold increase of the arterial flow compared with controls and a 6.7-fold increase when considering the loss of tissue mass. This is in line with the results of previous reports, demonstrating HABR directly after PBL (29) with restoration of liver blood flow due to a 666% increase of hepatic arterial flow (22).

The HABR-mediated arterialization of the portal blood-deprived liver induces partial but not complete sinusoidal capillarization, indicated by transition of sinusoidal endothelial cells into capillary-type endothelial cells without development of a basal membrane (34). Nonetheless, there is no information about how PBL affects hepatic microcirculatory function. Herein we demonstrate for the first time that deprivation of portal flow provokes a significant shutdown of \( \frac{80}{100} \) of the regularly perfused sinusoids within the initial 30 min after PBL. Within 60 min, however, there was an early recovery of functional sinusoidal density to \( \frac{50}{100} \), which did not change over the subsequent 14-day observation period. Interestingly, the initial sinusoidal perfusion failure was associated with significant tissue hypoxia and hepatocellular dysfunction. The HABR-associated arterialization, however, normalized the hepatocellular function and tissue oxygenation despite the persistent \( \frac{50}{100} \) reduction of sinusoidal perfusion density. Besides arterialization of the microvascular blood flow, sinusoidal dilation may have contributed to the observed normoxgenation of the parenchymal tissue.

The HABR-mediated arterialization may involve the action of nitric oxide due to increased eNOS production. Previous studies analyzing the role of nitric oxide synthesis isoforms in manifestation of portal hypertension could demonstrate that portal vein ligation induces systemic hyperdynamic circulation and extrahepatic portal hypertension (18, 27). This was associated with an increased expression of eNOS in the splanchic vasculature (18). By using gene knockout mice, Theodorakis and coworkers (27) confirmed that eNOS rather than iNOS is causative for the development of hyperdynamic circulation and portal hypertension. We herein now demonstrate that PBL is also associated with an increased expression of eNOS in the liver, which may sustain the HABR-mediated arterialization and tissue oxygenation after PBL.

A recent study has indicated that PBL induces intracellular stress proteins and immediate-early genes such as heat shock protein 70, heme oxygenase-1, and early growth response gene-1 (15). These genes are known to contribute to both

![Fig. 11.](http://ajpgi.physiology.org/)

**Fig. 11.** PCNA immunohistochemistry, indicating cellular proliferation, of a sham-operated liver (A) and a liver 14 days after PBL (B). Magnification, \( \times175 \). C: semiquantitative analysis of cells with PCNA-positive nuclei in controls and at days 3, 7, and 14 after PBL. Note the significant increase of the number of PCNA-positive nuclei at day 14 after PBL (B and C). Values are means \( \pm \) SE; \(*P < 0.05\) vs. control.

![Fig. 12.](http://ajpgi.physiology.org/)

**Fig. 12.** Expression of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) proteins. A: Western blot analysis for eNOS and iNOS after PBL. Analyses indicated that eNOS expression at day 14 was significantly increased to values that were threefold higher than that measured at day 0 (B). Analysis of iNOS expression revealed a slight but not significant decrease at days 3 and 7 after PBL and values at day 14 that were comparable with that measured at day 0 (C). Values are means \( \pm \) SE; \(*P < 0.05\) vs. control; \(\#P < 0.05\) vs. day 3; \(\$P < 0.05\) vs. day 7.
angiogenesis (24, 33) and tumor growth (13, 19, 26). Müller et al. (15) hypothesized that portal flow-deprived tissue up-regulates defense mechanisms like heme oxygenase-1 and heat shock protein 70 in response to local hypoxia, which are capable of stimulating neovascularization. In the present study, however, we could demonstrate tissue hypoxia only initially after PBL, which was followed by normalization of oxygenation. Accordingly, we could not observe angiogenesis and new vessel formation after PBL. In contrast, deprivation of portal flow was associated with sinusoidal remodeling, including an overall rarefaction of the absolute number of sinusoids. Besides the normalized parenchymal oxygenation, the induction of plasminogen activator inhibitor-1 (14) may have caused the lack of angiogenesis, because plasminogen activator inhibitor-1 is known to act in an antiangiogenic manner (6), counteracting the expression of the proangiogenic genes.

Previous reports (15, 16, 20, 25, 29) have demonstrated a pronounced reduction of weight, size, and number of hepatocytes at 5–8 days after PBL. The later course of the weight of the ligated lobe, however, was not monitored in these studies. The present study confirms the rapid weight reduction during the first few days after PBL. Interestingly, however, we demonstrated for the first time a recovery of the weight of the ligated lobe at day 14, indicating a late onset of proliferation and regeneration.

The mechanisms of liver atrophy after PBL are still controversially discussed. Some reports indicate that atrophy is dominated by necrotic cell death (2, 15). The first signs of necrosis could be observed after 12 h, resulting in confluent centrilobular necrosis in almost all acini at 24–48 h (15, 25). Necrotic cell death was thought to be followed by parenchymal apoptosis, as indicated by hepatocytes with dense, shrunken cellular bodies at 24–96 h after PBL (15). In contrast, others (20) suggested an early proapoptotic action with high Bax and low Bcl-2 expression, followed by late antiapoptotic conditions with low Bax and high Bcl-2 expression. Controversially, this pattern of Bax and Bcl-2 expression was associated with low caspase-3, -8, and -9 expression during the initial 48 h after PBL and an increase of these apoptosis-associated molecules after a 72-h period (20). All of these studies, however, are limited due to the 7-day restriction of the post-PBL observation period. Herein we demonstrate pronounced hepatocellular necrosis during the first 3 days after PBL, as indicated by increased liver enzymes and hepatocellular karyolysis. Although these signs of necrotic cell death successively vanished over time, nuclear condensation-associated late apoptotic cell death was detected.

There is little information on the capability of the ligated lobe to develop cell proliferation. Uemura and coworkers (28) demonstrated that negative regulators of hepatocyte proliferation, such as TGF-β1 and IL-1β, are strongly expressed in the ligated lobe during the first 7 days after PBL. Accordingly, we did not find PCNA expression during the initial 7-day period. However, the onset of late apoptotic cell death in the ligated lobe was accompanied by a marked increase of parenchymal proliferation, as indicated by a pronounced increase of PCNA-positive stained hepatocytes at day 14 after PBL. In fact, the late weight gain of the ligated lobe observed at day 14 indicates that the cell proliferation significantly dominated over the apoptotic and necrotic cell death. In consequence, this may explain the clinical findings that tumors within ligated lobes do not necessarily shrink in size but may present with acceleration of growth (3, 7).

This may occur despite significant apoptosis and necrosis. Overall, we demonstrate that there is an initial hypoxic injury (during the first few days after PBL) with mainly necrosis. The later phase of remodeling shows still some apoptosis; however, it is dominated by cell proliferation, as indicated by a >10-fold increase of PCNA staining from day 7 to day 14. Thus we would assume that a tumor in the same lobe would undergo some damage during the initial period after PBL (although most tumors are quite resistant against hypoxia); however, it would present with accelerated proliferation during the later phase of remodeling.

In conclusion, we demonstrate that PBL induces initial microcirculatory failure and tissue hypoxia in the portal flow-deprived liver lobe. This is counteracted by the onset of a HABR, which provokes arteriolarization, associated with normalization of tissue oxygenation, eNOS expression, and microvascular remodeling. The early microcirculatory dysfunctions have to be considered as the cause of liver atrophy, while the subsequent restoration of microvascular perfusion may represent the trigger for cell proliferation, indicating tissue repair and regeneration. Although we did not prove herein whether the mechanisms of hepatic microcirculation control after PBL are similar in tumor tissue compared with normal liver, we like to speculate that our findings may explain the accelerated tumor progression occasionally observed in patients after PBL.

ACKNOWLEDGMENTS

We thank C. Marx and J. Becker for excellent technical assistance.

GRANTS

This study was supported by a grant to the Medical Faculty of the University of Saarland (HOMFOR 2005: B/2004/20).

REFERENCES


