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Liver regeneration after different degrees of portal vein ligation

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ABSTRACT

Background: Selective portal vein ligation (PVL) is followed by ipsilateral atrophy and contralateral hypertrophy of the liver lobes. Although the atrophy-hypertrophy complex induced by PVL is a well-documented phenomenon, the effect of different degrees of extended portal vein occlusion on liver regeneration is not known. The aim of this study was to assess the effects of different degrees of portal occlusion on portal pressure and liver regeneration.

Materials and methods: Male Wistar rats ($n = 96$; 220-250 g) were randomized into three groups and underwent 70%, 80%, or 90% portal vein ligation, respectively. The portal pressure was measured immediately and 24, 48, 72, 120, and 168 h after PVL ($n = 6$/group/time point). The hepatic lobes and the spleen were weighed, and liver regeneration ratio was calculated. Changes in liver histology and the mitotic activity were assessed on hematoxylin-eosin stained slides.

Results: Higher degree of portal occlusion triggered a stronger regenerative response (regeneration ratio of PVL $70\%_{168h} = 2.23 \pm 0.13$, PVL $80\%_{168h} = 3.11 \pm 0.37$, PVL $90\%_{168h} = 4.68 \pm 0.48$) PVL led to an immediate increase in portal pressure, the value of which changed proportionally to the mass of liver tissue deprived of portal perfusion (PVL $70\%_{acute} = 17 \pm 2$ mm Hg, PVL $80\%_{acute} = 19 \pm 1$ mm Hg, PVL $90\%_{acute} = 26 \pm 4$ mm Hg). Findings in histology showed necro-apoptotic lesions in the atrophic liver lobes and increased mitotic cell count in the hypertrophic lobes. The mitotic cell count of PVL 90% peaked earlier and at a significantly higher value than of PVL 70% and PVL 80% (PVL $90_{24h}\%$: 96.0 ± 3.5 PVL $70\%_{48h}$: 64.0 ± 2.1, PVL $80\%_{48h}$: 56.3 ± 4.0). The mitotic index after 24 h showed a strong correlation with the acute portal hypertension.

Conclusions: A higher degree of portal vein occlusion leads to a greater regenerative response, presumably triggered by the proportional increase in portal pressure, which supports the role of the so-called "blood-flow" theory of PVL-triggered liver regeneration.

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Introduction

Hepatobiliary malignancies are rather common, and their incidence shows an increasing tendency worldwide. Primary liver cancer, which predominantly consists of hepatocellular carcinoma, is the sixth most common malignant tumor and the third leading cause of cancer related deaths.[1] However, liver malignancies are in most instances of secondary nature (metastatic liver tumors have approximately 20 times higher prevalence than primary tumors) and mostly provoked by underlying colorectal cancer.[2] Hepatectomy is the only curative option for long-term survival in patients with primary or secondary liver malignancies.[3] Technical advances have led to increased feasibility and safety of major liver resections; nonetheless, the rate of resectability is approximately 20%-30%, due to an insufficient volume of the remnant liver—also known as the future liver remnant—and the consequently increased risk of posthepatectomy liver failure.[4] In recent years, numerous surgical and interventional procedures have been developed to increase volume and function of the future liver remnant, of which portal vein occlusion (PVO) techniques (i.e., portal vein ligation—PVL or portal vein embolization—PVE) are the most widely used. The essence of these procedures lies in the artificially triggered atrophy of the liver lobes deprived of portal blood flow and the compensatory hypertrophy (liver regeneration) of the contralateral, abundantly perfused liver segments.[5]

The identity of key factors initiating liver regeneration after portal vein occlusion remains elusive. Nevertheless, cutting-edge research in recent years has led to the development of different hypotheses attempting to explain the underlying physiological trigger mechanisms. One prevailing concept is the so-called "blood-flow theory".[6] According to this notion, surgical ligation or embolization of portal branches results in a significant increase in the volume of portal flow per unit of liver mass in the nonoccluded lobes, which can lead to the initiation of the regeneration cascade by three alternate pathways. (1) Portal overflow results in elevated portal pressure, which reflects the amount of physical stress (shear stress) on the sinusoidal surface of the liver. The increased shear stress—stimulating the sinusoidal endothelial cells, hepatocytes, and Kupffer cells—triggers liver regeneration via mechano-chemical signal transduction.[7] (2) The accessibility of hepatotrophic factors (growth factors, hormones, and nutrients), transported by portal blood, increases in the nonoccluded liver lobes.[8] (3) Furthermore, portal venous hyperperfusion, accompanied by hepatic arterial hypoperfusion (due to hepatic artery buffer response), results in impaired liver tissue oxygenation. The impact of hypoxia on liver regeneration is still incompletely elucidated; however, it is assumed that relative hypoxia might activate adaptive mechanisms supporting the regenerative process.[9]

In clinical practice, tumor localization and dimensions determine the site of PVO, thus different degrees of portal vein occlusion lead to a highly variable extent of the excluded liver volume. Presumably, different degrees of PVO may impact portal pressure and hemodynamics of the nonoccluded liver lobes divergently; therefore, a difference in quantity and quality of the regenerative response seems highly probable. Despite the great clinical significance of this topic, we are not aware of any comparative studies investigating the effects of PVO on liver regeneration concerning occlusions involving various parenchymal masses above 70% in a standardized experimental setup.

The aim of the present study was to assess the effects of different degrees of PVO on portal pressure and liver regeneration in a standardized animal model and to evaluate a possible correlation between these parameters.

Methods

Animals

Male Wistar rats weighing 200-250 g (Semmelweis University, Central Animal Facility, Budapest, Hungary) were kept in a temperature-controlled and humidity-controlled environment with a 12-h light-dark cycle. Standard rat chow and water were provided *ad libitum*. All experiments were performed in accordance with the principles of the "Guide for the Care and Use of Laboratory Animals" guidelines (eighth edition, NIH Publication, 2011), and the protocol was approved by the Committee on Animal Experimentation of Semmelweis University (license number: PEI/001/313-4/2014).

Surgical procedure and experimental design

Surgical interventions were performed under general anaesthesia with intraperitoneal injection of ketamine (75 mg/body weight kg) and xylazine (7.5 mg/b.w. kg). After median laparotomy, the liver lobes were mobilized, and the median as well as the left lateral liver lobes were gently lifted, followed by cautious preparation of the hilar structures using an operating microscope (ZeisOpmi, Jena, Germany). The corresponding branches of the portal vein were ligated with a 6-0 braided silk suture (Atramat, Internacional Farmaceutica, S.A. De C.V., Coyoacán, Mexico) in accordance with the grouping order (see below). Great care using an atraumatic surgical technique was exerted to keep the hepatic arteries and bile ducts intact and so as to avoid hemorrhage. After surgery, the peritoneal cavity was closed in two layers by continuous suture, and the animals were returned to their cages.

Animals were randomly allocated to three experimental groups depending on the extent of PVL (Fig. 1.): In the PVL 70% group, complete ligation of the left primary portal branch supplying the median and left lateral lobes—corresponding to ~70% of total liver volume—was performed. In the PVL 80% group, in addition to the former procedure, a further ligation was performed to exclude the caudate lobes, leading to ligation of ~80% of total liver volume. In the PVL 90% group, the complete ligation of the right and left primary portal branches affected the right lateral, median, and left lateral lobes, corresponding to ~90% of total liver volume. Animals were reoperated 24, 48, 72, 120, and 168 hours after PVL (6 animals/time point/group, $n = 3 \times 5 \times 6$ [$\sum n = 90$]) to measure portal pressure; thereafter, animals were sacrificed in deep anaesthesia; during which liver and spleen were removed, weighed, and fixed for histopathologic evaluations. Animals in the