

Delayed Identification by CT of Parenchymal Changes Induced by Ligation of a Portal Vein Branch in the Rat- Preliminary Report

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ABSTRACT

AIM: To investigate the early and mid-term effects of ligation of a portal vein branch on liver structure and function and to correlate histological observations with images obtained with the use of computed tomography.

MATERIALS AND METHODS: Rats were randomly divided into 6 groups and subjected to the ligation of the left branch of portal vein. Liver damage was evaluated measuring aminotransferase levels, by histological analysis, proliferating cell nuclear antigen (PCNA) gene expression and changes in liver weight in sham-operated animals and at different time points after surgery. Computed tomography scans were performed at 6, 24 and 33 days after PVBL.

RESULTS: A progressive atrophy of the left hepatic lobe, characterized by cellular death and reduction in volume, started at the 8 h time point,

becoming significant after 48 h. A compensatory response of the right lobe progressively restored liver volume. Both reduction in the size of the left lobe and hypertrophy of the remnant lobes were detectable by CT scan at later time points, i.e. 24 and 33 days after surgery.

CONCLUSION: Histological changes induced by ligation of a portal vein branch occur at an early stage, preceding detection by radiologic imaging.

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Key words: Portal vein branch ligation; Liver hypotrophy; Computed tomography

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INTRODUCTION

Ligation or embolization of the portal vein is a currently used technique preceding resection of large portions of hepatic tissue, such as in the case of right lobe metastases^[1]. In these cases a compensatory hypertrophy of the left lobe is needed to provide sufficient hepatic mass to avoid hepatic insufficiency after right lobe hepatectomy. The timing of surgery is mostly dependent on computed tomography (CT)-based detection of hepatic hypertrophy, but the detailed dynamics of the balance between shrinking of the tissue fed by the ligated vessel, hypertrophy of the remaining liver, and CT findings has been only partially investigated.

The aim of this study was to investigate the early and mid-term effects of ligation of a portal vein branch (PVBL) on liver structure and function and to correlate histological observations with images obtained with the use of computed tomography.

METHODS

Animals

Male Sprague-Dawley rats (Harlan Nossan, Correnzana, Italy) weighing 175-200 g were used for these studies. All rats were kept in a temperature - controlled room with a 12 h light-dark cycle. Animals were fed a standard rat chow and fasted overnight before the experiment, with free access to water. Animals received humane care and all procedures were performed according to National guidelines and approved by the Institutional Animal Care and Use Committee.

Surgical procedures

All operations were carried out under general anesthesia obtained with administration of Zolazepam (Zoletil[®], 40 mg/kg body weight, intraperitoneum). A midline abdominal incision was performed, and the liver exposed. Under surgical microscopic guidance, the portal branch feeding the left liver lobe was isolated from the hepatic artery and bile duct, and completely ligated with a non-absorbable suture. In sham-operated animals, the same portal branch was isolated and manipulated with an identical procedure, but not ligated. Postoperatively, rats had free access to food and water. Rats were randomly divided into 6 groups of 3-4 animals each and sacrificed at 5, 8, 48, 96 or 144 hours following operation. The whole liver was weighed and the two lobes were then separated and re-weighed individually.

Aminotransferase activity

At the time of sacrifice, a blood sample was obtained by puncture of the inferior vena cava. Plasma levels of aspartate (AST) and alanine aminotransferase (ALT) were determined using a specific commercial kit (Diagnostic Systems, Holzheim, Germany).

Histology

Specimens of hepatic tissue obtained from both the left and right lobes were fixed in 4% buffered formaldehyde for 5 h and embedded in paraffin. Slides were then stained with haematoxylin and eosin for histological examination and subsequently examined by an expert in liver pathology blinded to the surgical procedures received by the animals.

Computed tomography

All examinations were carried out under anesthesia with Zoletil[®], as previously indicated, using a MDCT scanner (Sensation 64, Siemens Medical Systems, Erlangen, Germany) with the following settings: 80 kV, 200 mA, slice thickness 0.6 mm. CT scans were performed at 6, 24 and 33 days after PVBL. Control rats were subjected to CT scan 5 days after sham surgery.

RNA isolation and quantitative real-time PCR

Total RNA from liver tissue was isolated with an RNeasy mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Total RNA (5 µg) from each sample was reverse transcribed to complementary DNA (cDNA) using the SuperScript III first strand synthesis system (Invitrogen, Grand Island, NY, USA), according to the manufacturer's instructions. Hepatic mRNA gene expression was evaluated using quantitative real-time RT-PCR with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as housekeeping gene. Quantitative real-time RT-PCR and analysis were performed using a Corbett RotorGene 6,600 analyzer (Corbett Life Science, Sydney, Australia). FAM-labelled probes and specific primers were obtained as Assays-on-Demand (Applied Biosystems, Foster City, CA, USA). Relative gene expression was calculated as $2^{-\Delta Ct}$ ($\Delta Ct = Ct$ of the target gene minus Ct of GAPDH).

Statistical analysis

All results are expressed as mean±SD. Statistical analysis was performed using Student's *t*-test. *P* values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

We first evaluated serum aminotransferase levels following PVBL. While no changes in serum ALT or AST were observed in sham-operated animals, a detectable increase in liver enzymes was measurable as early as 5 hours after surgery (Figure 1A). Peak increase in ALT and AST levels was found 8 hours after PVBL, when liver enzymes were 4-fold higher than over control. These parameters returned to near-normal values at 48 hours and remained stable thereafter.

We then analyzed the weight of the whole liver and the one of the individual lobes in sham-operated and PVBL animals at different time points after surgery (Figure 1B). The contribution of the left lobe to total liver weight was approximately one third in control rats. This contribution started to decrease as early as 8 hours after surgery and rapidly declined thereafter, becoming about one fifteenth of the whole weight at 6 days after PVBL. At later time points it was no longer possible to detect the left lobe, as only very small remnants were visible opening the abdomen. Conversely, a progressive increase in the proportion of weight provided by the residual lobes was evident, becoming approximately 90% at 6 days compared to 70% in sham-operated animals. These data are compatible with the progressive hypertrophy of the residual lobes triggered by loss of hepatic mass of the left lobe (Figure 1B).

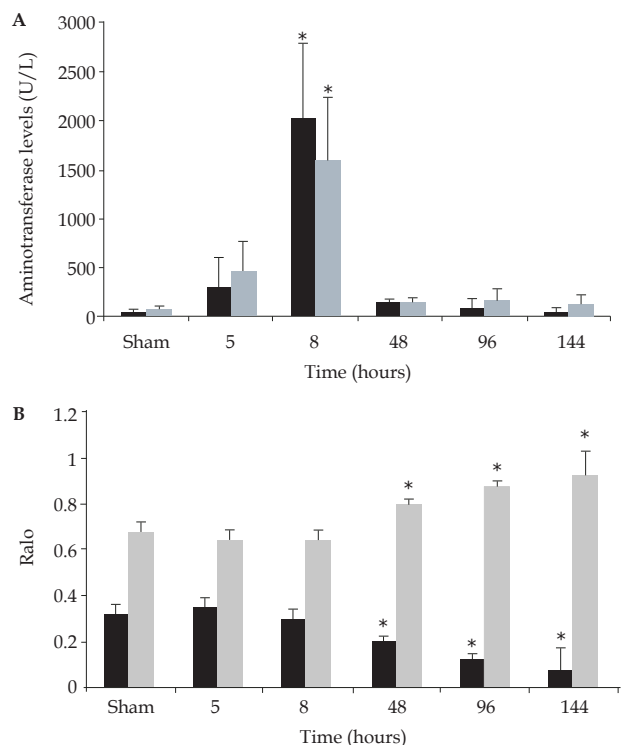


Figure 1 Time course of serum aminotransferase levels (A) and of changes of hepatic lobes weight (B) after portal vein branch ligation. A: Time course of serum alanine aminotransferase (ALT, dark grey bars) and serum aspartate aminotransferase (AST, light grey bars) levels following ligation of the left branch of the portal vein in rats. Data are given as mean±SD. **p*<0.05 vs sham; B: Time course of changes in the ratio of the weight of the left (black bars) and right (white bars) lobes to total liver weight after the ligation of the portal vein. Values are given as mean±SD. **p*<0.05 vs sham.

The proliferative response occurring after partial hepatectomy or acute or chronic injury is generally considered as a compensatory event to the reduction in functional liver mass^[2]. Another condition in which a similar response can be obtained is represented by the ligation of branches of the portal vein, which induces atrophy of the lobes deprived of portal blood. The hepatocyte proliferation starts in parallel to the appearance of atrophy, to restore the liver homeostasis after reduction of the hepatic mass^[3]. These events were confirmed by qRT-PCR analysis of hepatic mRNA gene expression of PCNA, involved in DNA replication in eukaryotic cells (Figure 2). Of note, the time course of changes in PCNA mRNA was similar to the one reported after partial hepatectomy^[4].

No apparent changes in hepatic architecture were found at light microscopy examination of control liver (Figure 3A). Analysis of the left lobe 8 hours following PVBL revealed inflammatory infiltration, characterized by polymorphonuclear leukocytes and sparse granulomas, associated with lamellar atrophy and large areas of necrosis in zone 3 of the hepatic acinus (Figure 3B). At later time points (48, 96 or 144 hours after surgery) necrotic areas enlarged. As cellular death was progressively more severe (Figure 3C).

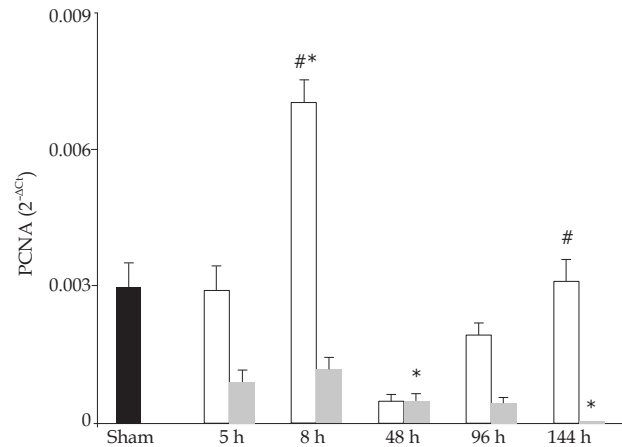


Figure 2 Time course of PCNA gene expression after portal vein branch ligation. At the time of sacrifice, liver tissue was collected for RNA extraction and the expression of PCNA at various time points after PVL was measured by qRT-PCR in sham operated rats (black column) and at different time points in the right (white columns) and left (gray columns) lobes. Relative gene expression was calculated as $2^{-\Delta Ct}$ ($\Delta Ct = Ct$ of the target gene minus Ct of GAPDH). * $P < 0.05$ vs Sham; # $P < 0.001$ vs left.

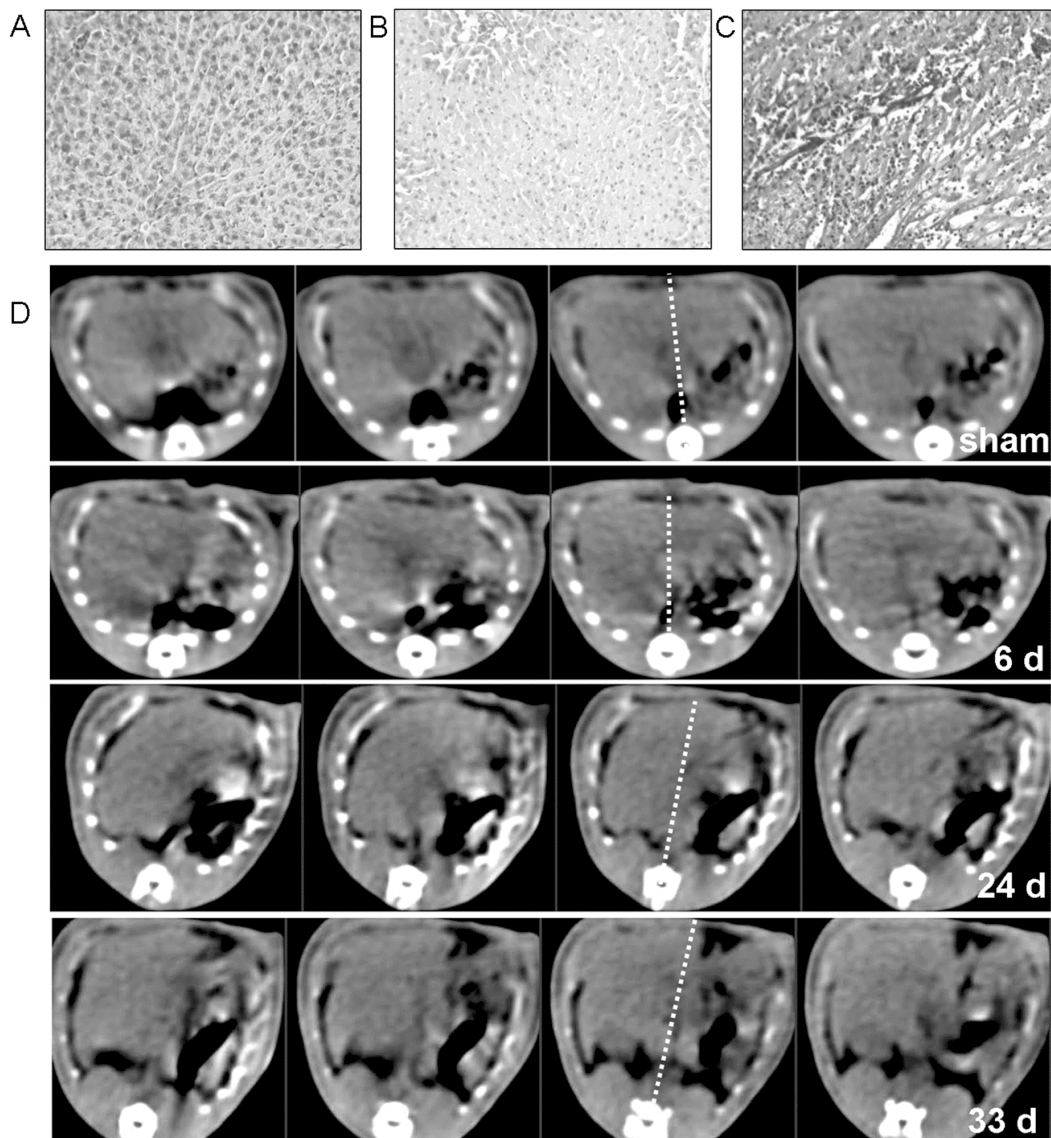


Figure 3 Histological sections (A, B, C) and CT images (D) of rat liver after portal vein branch ligation. A-C) Microphotographs of the liver sections from rats subjected to a sham-operation (A), and 8 h (B) or 48 h after the portal vein ligation (C). D) CT scans were performed in sham-operated rats or 6, 24, 33 days after surgery (D). Each row depicts adjacent slices obtained during the scan. The white dotted line divides the right from the left liver. The left lobe at the 6-day scan is similar to that observed in sham-operated animals. Conversely, a reduction in volume is appreciable at 24 days.

To correlate the biochemical, and macroscopic or microscopic changes in liver pathology with imaging, a CT examination was conducted at different time points. The size of the left liver lobe appeared to be unchanged at the 144 hour time point, compared to control, sham-operated animals (Figure 3D). A marked reduction in the size of left lobe was evident at the 24-day examination. The lobe was no longer evident at the CT scan performed 33 days after surgery (Figure 3D). At the same time, hypertrophy of the remnant lobes was not evident until the 24 day time point.

It might be important to emphasize that in human imaging this phenomenon can be recognized. In fact, the arterial buffer response leads to arterialization of the liver parenchyma whenever portal flow decreases^[5,6]. Due to the high oxygen tension and the low amount of nutrients, if these conditions are maintained the liver parenchyma undergoes metabolic changes that present as an area of hypodensity on imaging. This is likely due to the presence of edema, fibrosis and/or depletion (so called “metabolic infarction”) of hepatocytes in the underlying parenchyma^[7-9]. Based on these considerations, we expected to observe the same compensatory mechanisms in rats, although this was not detectable, at least evaluating the early events following PVBL. Additional studies, currently undergoing in our laboratories, are needed.

Our results confirm that significant events occurring after PVBL are evident shortly after surgery. In fact, a marked inflammatory infiltration and evidence of cellular death and hepatocyte loss is appreciable microscopically as early as 8 hours following ligation of the vessel. Of note, necrosis did not uniformly affect the hepatic lobule, but was more marked in zone 3. Additional evidence that liver damage is an early event after PVBL is provided by the observation that a marked (50-fold) and significant increase in serum ALT/AST levels is detectable at this same 8-hour time point. Shortly after, i.e. at 48 hours and later, progressive hypotrophy of the ligated lobe and compensatory hypertrophy of the remnant lobes, was detectable by directly weighing the different liver lobes and confirmed by PCNA gene expression. However, these changes induced by PVBL were not evident by CT scan until 24 days after surgery.

Although the limited spatial resolution of CT scans in a rat liver should be acknowledged, these results indicate that radiologic evidence of left lobe hypotrophy and remnant lobe hypertrophy is considerably delayed compared to pathologic data. These preliminary data may be helpful when programming resective surgery after portal embolization of a liver lobe. In fact, the clinician should be aware that changes observed at CT scan are likely to be not completely representative of the pathologic changes occurring within the liver.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests and received no financial support.

REFERENCES

- 1 Heinrich S, Jochum W, Graf R, Clavien PA. Portal vein ligation and partial hepatectomy differentially influence growth of intra-hepatic metastasis and liver regeneration in mice. *J Hepatol* 2006; **45**(1): 35-42
- 2 Lambotte L, Li B, Leclercq I, Sempoux C, Saliez A, Horsmans Y. The compensatory hyperplasia (liver regeneration) following ligation of portal branch is initiated before the atrophy of the deprived lobes. *J Hepatol* 2000; **32**: 940-945
- 3 Rozga J, Jeppsson B, Bengmark S. Portal branch ligation in the rat. Reevaluation of a model. *Am J Pathol* 1986; **125**: 300-308
- 4 Assy G, Gong Y, Zhang M, Pettigrew M, Pashniak D, Minuk GY. Use of proliferating cell nuclear antigen as a marker of liver regeneration after partial hepatectomy in rats. *J Lab Clin Med* 1998; **131**: 251-256
- 5 Carmichael FJ, Saldivia V, Varghese GA, Isreal Y, Orrego H. Ethanol-induced increase in portal blood flow: role of acetate and A1-and A2- adenosine receptors. *Gastrointestinal Liver Physiology* 1988; **18**: G417-G423
- 6 Kawasaki T, Carmichael FJ, Saldivia V, Roldan L, Orrego H. Relationship between portal venous and hepatic arterial blood flows. Spectrum of response. *Am J Physiol* 1990; **259**: G1010-G1018
- 7 Colagrande S, Pradella S, Lucarini S, Marra F. Transient hepatic parenchymal enhancement detected at dynamic imaging: A short instruction manual for the clinician. *Digestive and Liver Disease* 2012; **44**: 363-368
- 8 Colagrande S, Carmignani L, Rosselli G, Villari N. Arterial hepatic sectorial enhancement (ASE) as a sign of acute portal thrombosis. Three case reports. *Radiol Med* 1998; **96**: 639-641
- 9 Ternberg JL, Butcher HR Jr. Blood flow relation between hepatic artery and portal vein. *Science* 1965; **150**: 1030-1031

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