

## Chronological changes in the liver after temporary partial portal venous occlusion

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### Abstract

**AIM:** To investigate time-dependent changes caused by temporal portal vein obstruction and subsequent reperfusion in the lobe with or without an occluded portal vein.

**METHODS:** The portal vein (PV) of the anterior lobe of the liver of a male Wistar rat (8 wk-old) was obstructed (70%) for 12, 24, 36 and 48 h, respectively, and models were sacrificed at 48 h after reperfusion (each group:  $n = 10$ ). The histological changes and the status of liver regeneration were compared between a liver biopsy performed on each lobe after temporary obstruction of the portal vein in the same rat liver, and the liver extracted at the time of sacrifice (48 h after reperfusion).

**RESULTS:** With regard to the obstructed lobe, the liver weight/body weight ratio significantly decreased according to obstruction time. On the other hand, in the

non-obstructed lobe, there were no significant differences within each group. The duration of PV occlusion did not seem to be strong enough to introduce liver weight increase. Stimulation of liver regeneration was brought about in the non-occluded lobe by 12-h occlusion, and was sustained even at 48 h after reperfusion. The obstructed lobe atrophied with the passage of time in the obstructed state. However, the proliferating-cell nuclear antigen labeling index also increased at 48 h after reperfusion, and a repair mechanism was observed.

**CONCLUSION:** Temporary blood flow obstruction of the portal vein may become a significant trigger for liver regeneration, even with an obstruction of 12 h.

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**Key words:** Temporary; Portal vein; Occlusion; Regeneration; Liver

**Core tip:** This paper describes the chronological effects of temporary portal venous branch ligation on liver regeneration in rats. These results imply that, in the future, it might be possible to control liver regeneration. In the clinical setting, we have just completely occluded the portal venous branch irreversibly.

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### INTRODUCTION

Permanent obstruction of the portal vein, as clinically

applied in portal branch ligation (PBL) or percutaneous transhepatic portal venous embolization, evokes liver regeneration<sup>[1-4]</sup>. This technique enables relatively major hepatic resection for malignancy in an occluded liver lobe<sup>[5-9]</sup>. In addition, PBL has been used to induce a regenerative stimulus for transplanted hepatocytes or pancreatic islet cells for cell therapy<sup>[10,11]</sup>.

Although short term temporary occlusion can induce some degree of liver regeneration, an investigation of liver regeneration caused by temporary portal vein obstruction, as well as time-dependent changes resulting from reperfusion, has not yet been performed<sup>[12]</sup>. Therefore, the current study aimed to examine time-dependent changes in a lobe with a portal vein occlusion of an unobstructed portal vein caused by temporary portal vein obstruction and reperfusion as the central focus.

## MATERIALS AND METHODS

### Animals

Male Wistar rats (200-240 g, Japan SLC Inc., Shizuoka, Japan) were used for the experiments. All animals were maintained at 24 °C with a 12-h light-dark cycle and given free access to tap water and standard laboratory chow. The animals were treated in accordance with the guidelines stated in the University of Nagasaki Research Animal Resources during all experimental procedures.

### Experimental design

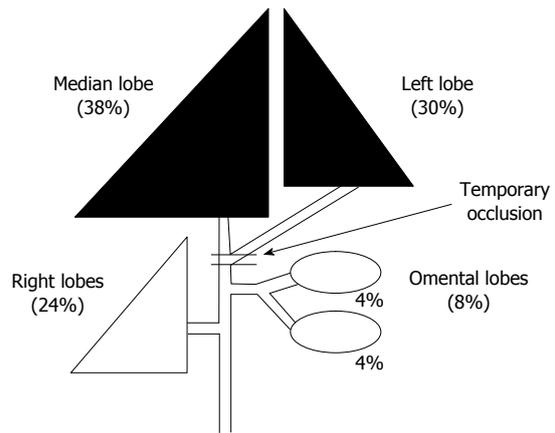
The portal vein of the anterior lobe (medial and left lobes) of the liver of a male Wistar rat (8 wk old) was occluded (70%) for 12, 24, 36 and 48 h, respectively (Figure 1). Rats to be sacrificed were prepared at 48 h after each reperfusion (models for each group: *n* = 10, Figure 2). The histological changes over time and the status of liver regeneration were compared between liver biopsies performed from each lobe after temporary obstruction of the portal vein in the same rat liver, and the liver extracted at the time of sacrifice (48 h after reperfusion).

### Liver to body weight ratio

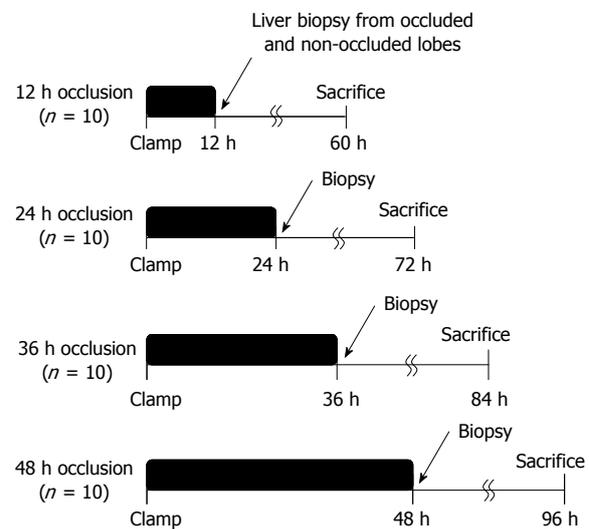
The body weights and liver weights were recorded following the sacrifice of the rats to compare the rate of liver regeneration. The liver weight was expressed as a percentage of the body weight (%) and used as an index.

### Histology and immunohistochemistry

Formalin-fixed paraffin embedded (4 μm) sections were used for hematoxylin-eosin (HE) staining. Proliferating-cell nuclear antigen (PCNA) immunostaining was performed to examine hepatocyte proliferation using a mouse monoclonal antibody against PCNA (clone-PC 10; Dako, Kyoto, Japan)<sup>[13]</sup>. Briefly, liver tissue specimens were fixed in 10% buffered formalin, embedded in paraffin and then cut into 5 μm sections. The deparaffinized sections were heated in a microwave three times in phosphate-buffered saline (PBS) for 5 min each and were then washed three times with PBS for 5 min each. After blocking endogenous peroxidase activity, the specimens



**Figure 1 Schematic drawing of the rat model.** The portal vein of the anterior lobe (black area) of the liver was occluded (68%) for various durations, while the arteries remained open.



**Figure 2 Experimental protocol.** The portal vein of the anterior lobe (median and left liver lobes) of the liver of a male Wistar rat was occluded (70%) for 12, 24, 36 and 48 h. Rats to be sacrificed were prepared at 48 h after each reperfusion (models for each group: *n* = 10).

were washed three times with PBS for 5 min each. The sections were incubated with an antibody against PCNA overnight at 4 °C. After washing several times with PBS, biotin-labeled secondary antibody was added for 1 h at room temperature. After washing several times with PBS, the tissue peroxidase activity was visualized using diaminobenzidine.

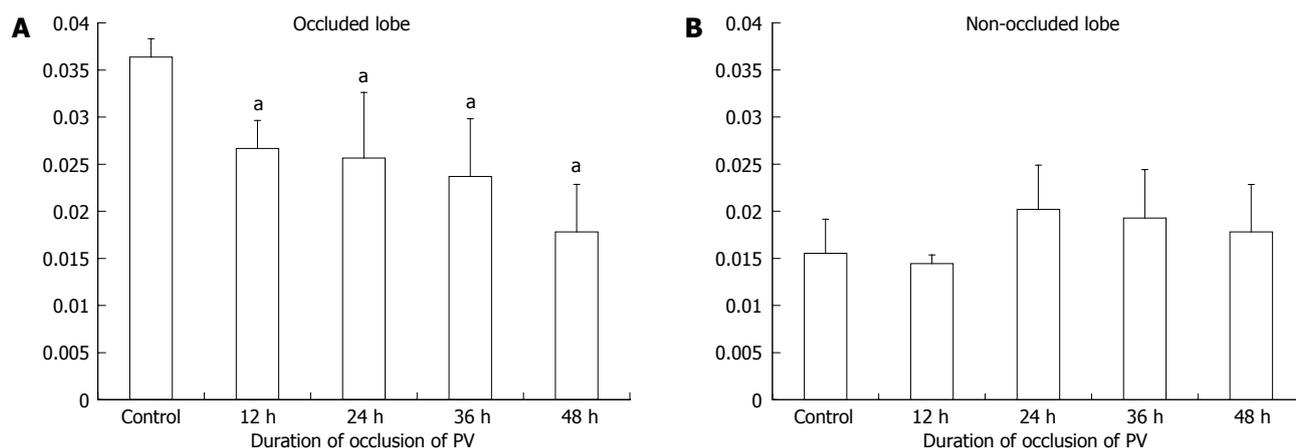
The PCNA labeling index (PCNA LI) was then determined as the number of PCNA-positive cells among 1000 counted cells.

### Statistical analysis

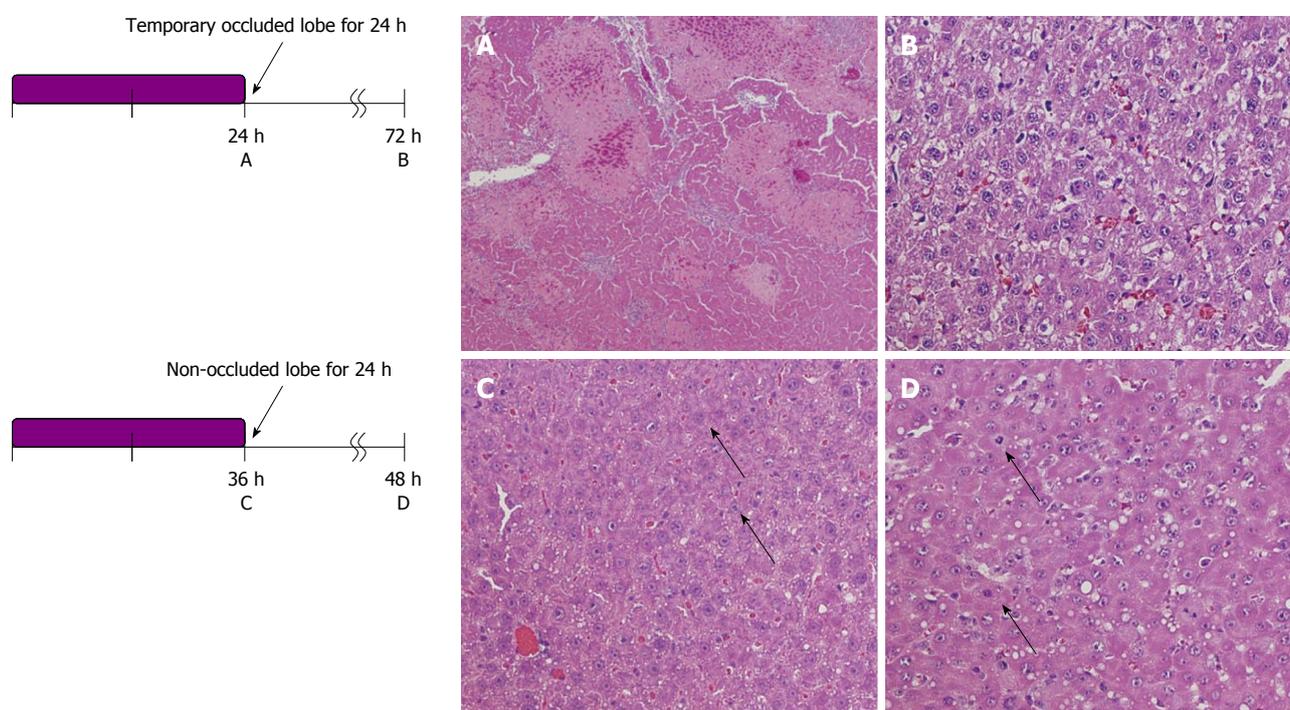
All of the data were expressed as the mean ± SD. The Mann-Whitney *U*-test was used for data analysis. A level of *P* < 0.05 was considered statistically significant.

## RESULTS

With regard to the obstructed lobe, the liver weight/body



**Figure 3 Liver weight/body weight.** A: The liver weight/body weight ratio was decreased by temporary occlusion of the occluded anterior lobes; B: There were no significant differences within each group in non-occluded lobe. <sup>a</sup>*P* < 0.05 vs control group.



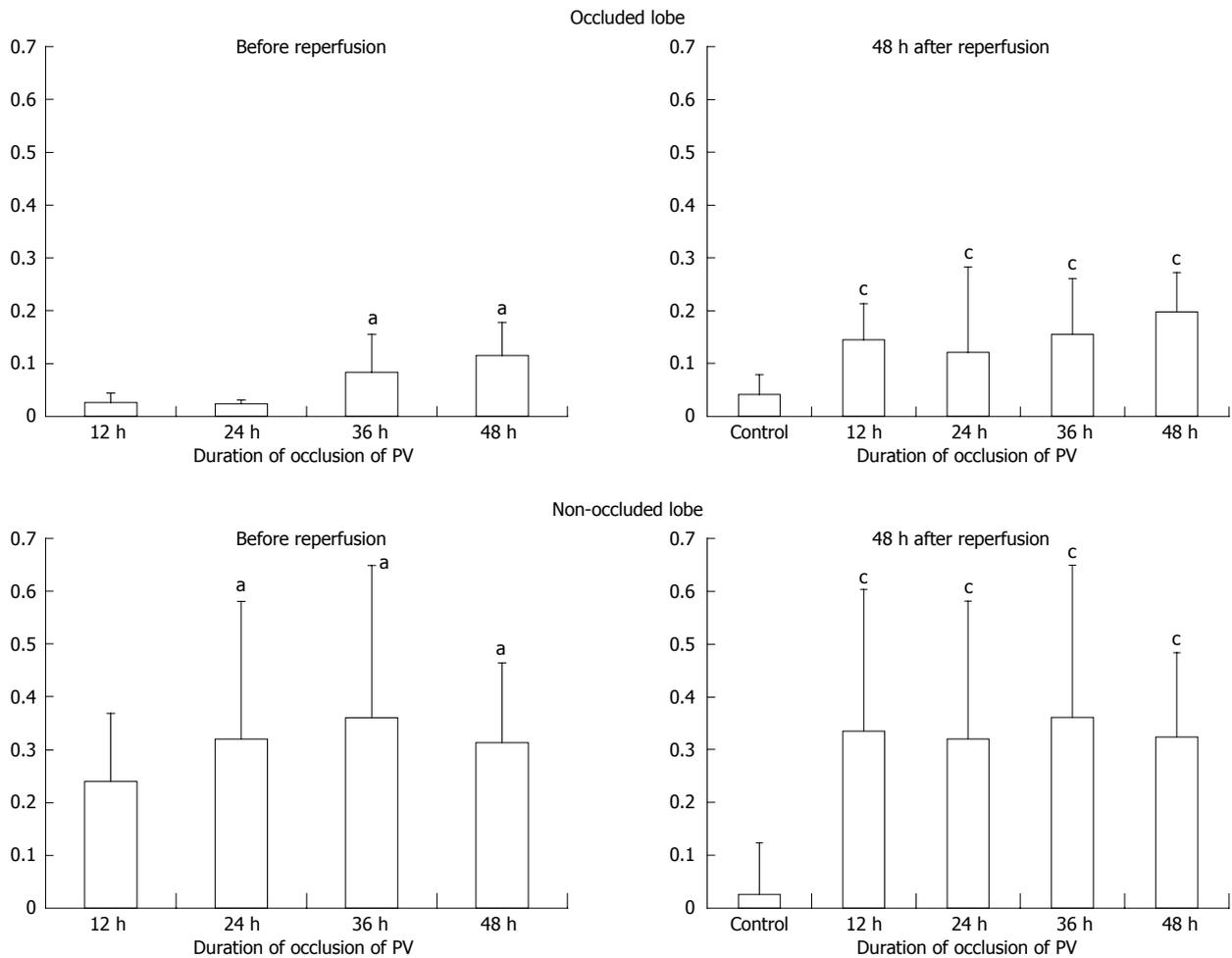
**Figure 4 Changes in hepatic histology.** Histology in temporary occluded lobes. A: 24 h occlusion, × 100; B: 36 h occlusion, × 400; C: In non-occluded lobes 24 h occlusion, × 100; D: 48 h occlusion, × 400 is shown by HE staining. In the occluded liver lobe, before reperfusion, coagulative necrosis may be observed around the central vein in proportion to the occlusion time. However, the above-mentioned necrotic area decreased at 48 h after portal vein reperfusion. In the non-occluded liver lobe, hepatocytes became hypertrophic, and some mitoses could be observed (arrows).

weight ratio significantly decreased with increasing obstruction time (Figure 3). On the other hand, in the non-obstructed lobe, there were no significant differences within each group. The duration of PV occlusion did not seem to be strong enough to induce an increased in liver weight.

Liver histology was investigated under HE staining (Figure 4). In the occluded liver lobe, before reperfusion, coagulative necrosis was observed around the central vein in proportion to the occlusion time. However, after 48 h of reperfusion, the above-mentioned necrotic area

decreased. On the other hand, in the non-occluded liver lobe, hepatocytes became hypertrophic, and some mitoses were observed.

In the non-obstructed lobe, there were no significant differences in the PCNA LI within each group (Figure 5). LI seemed to peak at 36 h of biopsy (non-obstructed models at 12, 24, 36 and 48 h = 24%, 32%, 36% and 31%, Figure 5C). In the non-occluded lobe at 48 h after reperfusion (models for each group = 33%, 32%, 36% and 32%), the PCNA LI was still significantly increased at all points compared with the control (Figure 5D).



**Figure 5 Proliferating-cell nuclear antigen labeling index.** Proliferating-cell nuclear antigen (PCNA) labeling index in each lobe at the time before reperfusion and 48 h after reperfusion. <sup>a</sup>*P* < 0.05 vs 12 h group; <sup>c</sup>*P* < 0.05 vs 24 h group. PV: Portal vein.

On the other hand, hardly any positive cells were observed in a biopsy of the obstructed lobe (obstructed models at 12, 24, 36 and 48 h = 2%, 2%, 8% and 10%). However, at 48 h after reperfusion, an increase in LI was observed (models for each group = 14%, 12%, 15% and 19%).

## DISCUSSION

Portal venous branch ligation or embolization (PBL or PBE) can induce atrophy of the ligated lobe, while inducing hypertrophy of a non-ligated lobe, which enables extended hepatectomy for a malignant tumor in a ligated lobe<sup>[14-16]</sup>. In addition, PBL has been used as a regenerative stimulator to induce transplanted cell proliferation in animal models for hepatocyte-based cell therapy<sup>[17-23]</sup>. The length of time of occlusion needed to induce remnant liver regeneration, *i.e.*, temporary portal venous occlusion, remains unknown. In the present study, the stimulation of liver regeneration brought about in the non-occluded liver lobe was sustained, even after 48 h from reperfusion. Thus, temporary blood flow obstruction of the portal vein may be a significant trigger of liver regeneration, with an obstruction of at least 12 h. However, the

duration of PV occlusion in this study did not seem to be long enough to induce an increase in liver weight.

Interestingly, there was no significant difference in PCNA LI in the non-occluded lobe according to the duration of portal vein occlusion up to 48 h. Therefore, in the clinical setting, the same extent of liver hypertrophy may be induced with temporary balloon occlusion in as short as 12 h, to minimize an invasive procedure, although there might be difference among species.

As a cell therapy, many investigator have used liver for the engraftment of many cell types<sup>[10-12,14-16]</sup>. In fact, transplanted cells (hepatocytes, pancreatic islet cells or genetically engineered cells) could be induced to proliferate using temporary portal venous occlusion. Although there must be some differences between humans and rodents in terms of liver regenerative activity, our results provide a new insight into temporary stimulation of liver regeneration for subsequent treatment procedures<sup>[24-26]</sup>.

On the other hand, the PCNA labeling index of the obstructed lobe was also increased 48 h after portal venous reperfusion. This could be a repair mechanism for portal vein ischemia in the occluded lobe, although PCNA LI was lower compared to that in the non-occluded lobe that undergoes liver regeneration<sup>[14]</sup>. Although it

was not observed up to 48 h, this result of the temporary occlusion of the lobe provided an interesting phenomena; however, the lack of temporal portal venous flow could become atrophic if portal venous ischemia had lasted longer. The duration for the “point of no return” should be investigated in further research.

In conclusion, a temporary blood flow obstruction of the portal vein may be a significant trigger for liver regeneration, even with an obstruction of 12 h. The histological changes in the unobstructed lobe and obstructed lobe in cases of temporary blood flow obstruction of the portal vein and at 48 h after reperfusion were described.

## COMMENTS

### Background

Permanent obstruction of the portal vein, as clinically applied in portal branch ligation (PBL) or percutaneous transhepatic portal venous embolization, evokes liver regeneration. This technique enables relatively major hepatic resection for malignancy in an occluded liver lobe. In addition, PBL has been used to induce a regenerative stimulus for transplanted hepatocytes or pancreatic islet cells for cell therapy.

### Research frontiers

Portal venous branch ligation or embolization can induce atrophy of the ligated lobe, while inducing hypertrophy of a non-ligated lobe, which enables extended hepatectomy for a malignant tumor in a ligated lobe. In addition, PBL has been used as a regenerative stimulator to induce transplanted cell proliferation in animal models of hepatocyte based cell therapy. The length of time of occlusion required to induce remnant liver regeneration, *i.e.*, temporary portal venous occlusion, remains unknown.

### Innovations and breakthroughs

The histological changes and the status of liver regeneration were compared between a liver biopsy performed on each lobe after temporary obstruction of the portal vein in the same rat liver, and the liver extracted at the time of sacrifice (48 h after reperfusion).

### Peer review

This research is very important because it shows that a temporary obstruction of the portal vein as a trigger of liver regeneration. Recently, it has been used as a regenerative stimulator to induce transplanted cell proliferation. Thus, the manuscript approached an interesting subject from the surgical and scientific points of view; however, several aspects should be better evaluated.

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