

An Abnormal Proliferation at Day Four on 70% Partial Hepatectomy of Rats

Zenei Taira, Maya Shiraishi and Yukari Ueda

Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan

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Abstract: Restoration began during the latter part of the first day following the removal of 70% of the liver, and the preoperative ratio of the liver weight to the body weight was essentially restored from the tenth to the fourteenth day. The more rapid rate of restoration took place during the third and fourth days. Since transient loss of body weight ensued on operation and was not essentially recovered until from twenty-five to twenty-eight days, hepatic restoration was determined as complete at this time.

Key words: Basic research, 70% partial hepatectomy, hepatic restoration

Introduction

The liver is capable of recovering from damage or loss of its mass by means of proliferative activity, restoring it to normal size. This liver regeneration involves various growing processes that triggers, modulates, and stops cell proliferation. Thus, the experimental model has been examined regarding restoring after tissue injury or its loss. The mechanism of hepatic regeneration has been studied from various aspects. Detailed technology of 70% partial hepatectomy (PH) has been described by Higgins and Anderson (1933). They have reported that the weight of a regenerating liver reaches a minimum at 24 hours after a partial hepatectomy, and thereafter the weights gradually increases to reach the original weight by ten days.

In this study, we described further detailed regeneration process of the liver weight after the partial hepatectomy.

Materials and Methods

Chemicals

Collagenase, propidium iodide (PI), DMSO and Evans blue (EB) were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan. Other reagents were purchased from commercial sources, and were of the highest grade available.

Animal Treatment.

Eight-week-old male Donryu rats were obtained from the SLC Co., Ltd. (Shizuoka, Japan). Animals had free access to food and water during the experimental period. Some rats were given an i.p. injection of CCl_4 at 0.2 ml/kg, and then blood was drawn 24 hours later from the tail vein. Serum ALT activity was measured spectroscopically using a diagnostic kit from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

The liver resection of partial hepatectomy

Rats were anesthetized with ether, and the abdominal cavity was opened via a midline incision. After transection of the suspensory ligaments and central ligature, the median and left lateral lobes were removed, as described previously by Higgins and Anderson (1931). This is classically recognized as partial hepatectomy in the rat. In a separate experiment, the wet weights of resected liver and regenerating liver were measured to evaluate regeneration during 0 to 21 days after partial hepatectomy. The recovery ratio of regeneration liver was calculated by estimating that 70% of the total liver was resected by the operation. The

regenerating ratio R in liver after hepatectomy was calculated as following, $R=100 \times 0.7 \times (W2/W1)$, where W1 is weight the liver resected by the operation, and W2 is the weight of regenerating liver.

Pharmacokinetics of EB

Rats anesthetized with pentobarbital sodium were chronically tubing. EB (1 mg/kg) was administered intravenously and blood was usually collected. EB was determined by the absorbance method at 620 nm. The pharmacokinetic parameters, the mean residence time (MRT), the volume of distribution (V_{dss}) at steady state and total areas under the plasma concentration-time curve (AUC) were calculated using the moment analysis of plasma concentration-time curves.

Isolation of hepatocytes

The rats were anesthetized with 30 mg/kg of pentobarbital sodium and liver cells were isolated by the collagenase perfusion method. Briefly, the portal vein was cannulated with a polyethylene tube, and the superior vena cava was cut for drainage. Immediately after cannulation, 100 ml of 5 mM EGTA-Hepes buffer solution was perfused for about 5 min followed by 200-250 ml of 0.05% collagenase-Hepes buffer solution to disperse the tissue. The liver was then removed and dispersed with surgical blades in cold Ca^{2+} - and Mg^{2+} -free PBS(-) five times to isolate the hepatocytes.

Cell cycle

Cells were fixed by adding 700 μl of ice-cold 95% ethanol and vortexing. The cells fixed were centrifuged for 5 min and at 4°C for 5 min, and washed twice with 200 μl cold PBS. The cells were incubated in 1 ml RNase solution for 30 min at 37°C for 10 min, and washed with 200 μl PBS. Store in 200 μl PBS at 4°C until measurement. Immediately before flow cytometric analysis, the cells were incubate in 200 μl of PI solution for 30 min at room temperature and centrifuged for 50 min at 824g and 4°C. Resuspend in 200 μl PBS and store on ice shielded from light. To analyze the sample, transfer 100 μl of

Analysis of VEGF mRNA by RT-PCR

Livers were removed from rats 0, 1, 2, 3, 4, 5, 6 days after the operation, and frozen in liquid nitrogen. Total RNA was extracted from frozen livers using by the acid-guanidium thiocyanate-phenol-chloroform extraction method. The expression levels of vasculogenesis factors, VEGF and ANG, were estimated using by RT-PCR. The PCR conditions were 94°C for 10 sec, 55°C for 20 sec, and 72°C for 20 sec for 30 cycles. Amplified PCR products were electrophoresed, stained with EtBr and photographed. Oligonucleotide primer sequences used as follows;

Corresponding author: Taira Zenei, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan. Tel: +81 088 622 9611 ext 5711, Fax: +81 088 655 3051, E-mail: tairaz@ph.bunri-u.ac.jp

- 1) VEGFD:Sense 5'-gacatgctgt gggatacac caaatga-3'
 Antisense 5'-gtatttctta gaggccaac gtctg-3'
 2) ANG: Sense 5'-ataaccagtc agaggcagta catgctg-3'
 Antisense 5'-caagggccgg atcatcatgg tggtg-3'

The significance of differences between two mean values was measured using Student's t-test. A difference was considered to be significant at a p value of 0.05 or less.

Results

1. Recovery and ratio of liver regeneration after PH in rats To evaluate regeneration of liver in the rats after partial hepatectomy, we measured the regenerating ratio calculated using the wet weights of resected livers and regenerating livers in a separate experiment.

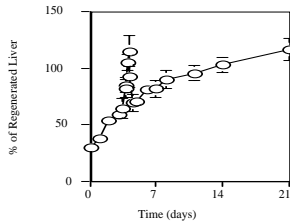


Fig. 1. Ratio of liver regeneration after HP in rats

The livers in surgery rats were recovered to the original liver weight for 14 days. The proliferation gradually increased, except the fourth day after PH. The abnormal proliferation began 90 hours after the surgical operation, reached 96 hours to a peak at ca. 100%, and recovered by 99 hours.

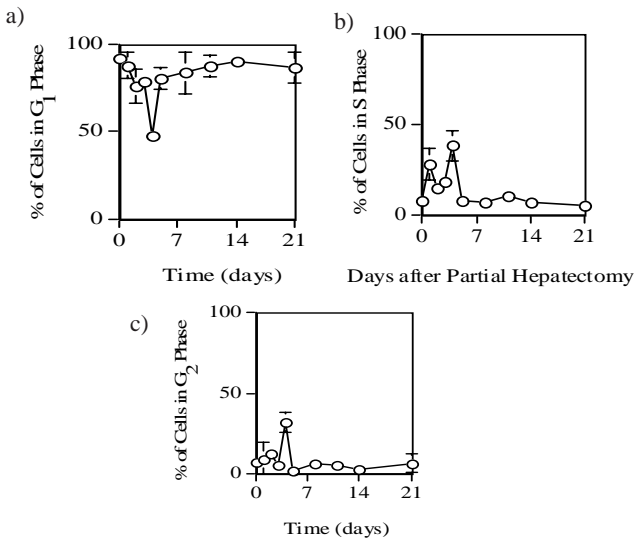


Fig. 2. Phases of the cell cycles on liver regeneration after PH

In this study, we demonstrated what happened at the day. In generally, it has been known that the hepatic regeneration repeats synchronously the proliferation. Thus, we examined the genetical change after PH. As shown in Fig. 2, hepatocytes synchronously proliferated so that cells in the S phase strikingly increase at 24 and 96 hours after the operation. This might be evidence that the abnormal proliferation results in the activation of the cell division. Furthermore to characterize physiologically liver regeneration after PH, we estimated pharmacokinetically change of blood volume using distribution volume of EB.

Change of distribution volume after HP

Fig. 3 shows the time-courses of plasma concentrations of EB administered intravenously at a dose of 10 mg/kg of EB to rats at

various days after PH, and then analyzed using the moment analysis. The result showed that the plasma volume Vdss increases to a peak at four days after the operation, and then recovers to the normal volume.

Vascular endothelial growth factors

As shown in Fig. 4, no clearly detectable band for VEGF mRNA could be found before PH, but the expression of VEGF mRNA increased markedly between 48 and 90 hours after PH, and thereafter decreasing gradually.

These results might show that the cell division and vasculogenesis cause jointly the abnormal liver generation at day 4.

To characterize changes in the hepatic function after PH, we measured the serum ALT values, in which PH rats were ip administered CCl₄ at 0.2 ml/kg and measured the serum ALT values 24 hours latter. As shown in Fig. 5, the serum ALT activity decreased significantly several % to the control value at 1 day

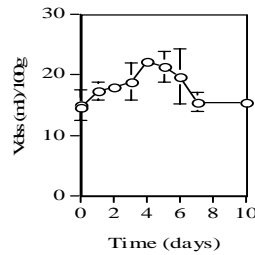


Fig. 3. Blood volume EB (1 mg/kg) was administered intravenously and blood was collected. EB was determined by the absorbance method.

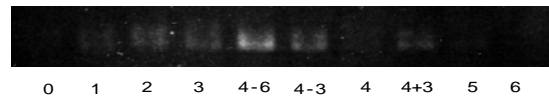


Fig. 4. Expression levels of mRNA for calcium binding proteins after CCl₄ administration

after PH, then recovered temporarily day 4 the control level, and did entirely after day 10. Thus, this might show that the liver function also recovers at day 4

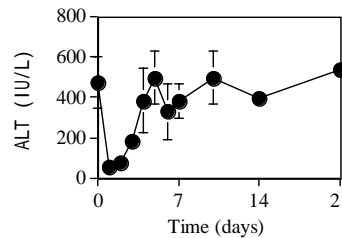


Fig. 5. Time-courses of serum ALT in PH rats 24 hours after CCl₄ administration

Discussion

Restoration began during the latter part of the first day following the removal of 70% of the liver, and the preoperative ratio of weight of the liver to weight of the body was essentially restored at from the tenth to the fourteenth day. The more rapid rate of restoration took place during the third and fourth days. Since transient loss of body weight ensued on operation and was not essentially recovered until after from twenty-five to twenty-eight days, hepatic restoration was determined as complete at this time.

References

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