Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 352 (2007) 311–316
BBRC
www.elsevier.com/locate/ybbrc

# Is there any effect of renal failure on the hepatic regeneration capacity following partial hepatectomy in rats?

Toru Kawai, Yukihiro Yokoyama *, Masato Nagino, Tomomi Kitagawa, Yuji Nimura

*Division of Surgical Oncology, Department of Surgery, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan*

Received 1 November 2006
Available online 13 November 2006

**Abstract**

The effects of renal dysfunction on liver regeneration capacity have not been fully elucidated before, although many patients with renal failure are subjected to hepatectomy due to hepatobiliary diseases. In this study, we sought to determine the effects of renal dysfunction on the hepatic regeneration capacity using rat chronic renal failure model. After establishing chronic renal failure (CRF group) by semi-total renal resection, the rats were subjected to 70% partial hepatectomy (PHx). Rats without renal failure were used as control (Sham group). The hepatic regeneration rate, histology of the liver, clearance of indocyanine green into the bile, and the expression of hepatic regeneration-associated genes in the liver were evaluated. The hepatic regeneration rate was lower in CRF group as compared to Sham group on day 1 after PHx. Mitotic index evaluated by histologic examination on day 1 after PHx was also significantly lower in CRF group. However, no difference in these indices was observed on day 2 and 7 between Sham and CRF. Indocyanine green clearance rate was almost identical between Sham and CRF on day 7 following PHx. The baseline expressions of the hepatic regeneration-associated genes, such as IL-6, TNF-α, HGF, c-fos, and c-jun, in the liver of CRF were significantly lower than those of Sham. However, the rate of upregulation of these genes was not significantly different between Sham and CRF. These results clearly demonstrate that the renal dysfunction, although initially delayes the onset, does not suppress the total hepatic regeneration capacity following partial hepatectomy. The function of the regenerated liver on day 7 after PHx also was not different. Our results provide a possibility that the hepatectomy can be indicated even for the patient with a chronic renal failure.


*Keywords:* Renal dysfunction; Partial hepatectomy; Subtotal nephrectomy; Mitotic index; Immediate early gene; IL-6; c-fos; Indocyanine green

Hepatic regeneration capacity is modulated by several factors including biliary obstruction [1], chronic viral infection [2], diabetes mellitus [3], etc. Due to these deteriorating factors, patients who underwent major hepatectomy occasionally encounter a critical hepatic failure because of an insufficient functional remnant liver volume.

Recent advancement of surgical technique and perioperative management enabled major hepatectomy for patients with severe chronic diseases. Chronic renal failure (CRF) has been thought to be one of the major risk factors for perioperative management of hepatectomy [4]. Patients with chronic renal dysfunction are complicated with coagulopathy [5], systemic atherosclerosis [6], and compromised immune function [7], all of which may increase the risk of major hepatectomy. Nonetheless, recent report by Sawada et al. showed that hepatectomy for patients with nonuremic minimal renal failure [8] can be safely performed with adequate indications, appropriate operative procedures, and perioperative management. Another report by Cheng et al. also showed the safety of hepatectomy in patients with end-stage renal failure [9]. However, these reports are retrospective clinical review and no well-controlled prospective study has been performed to determine the role of renal failure on the outcome of hepatectomy in patients with renal dysfunction. Moreover, there is no report, which

* Corresponding author. Fax: +81 52 744 2230.
*E-mail address:* yyoko@med.nagoya-u.ac.jp (Y. Yokoyama).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.11.011

evaluated the effects of renal dysfunction on the hepatic regeneration capacity following major hepatectomy. To answer this question, we used an animal chronic renal failure model made by 5/6 nephrectomy [10]. The regeneration rate of the liver following hepatectomy, mitotic index in the liver histology, indocyanine green clearance rate on day 7 after the hepatectomy, and regeneration-associated gene expression in the liver were evaluated and compared between renal failure and sham group.

## Materials and methods

*Animal and surgical procedure of CRF and 70% partial hepatectomy*. Male Wister rats (250–280 g) were purchased from SLC (Tokyo, Japan). The animals were kept in a temperature- and humidity-controlled environment in a 12-h light–dark cycle and they were allowed free access to water and diet at all times. All rat experiments were approved by the University Committee on Animal Research and received humane care in accordance with NIH publication 86-23 "Guide for the Care and Use of Laboratory Animals." They were randomly assigned to the CRF and Sham groups. The animals assigned to the CRF group underwent 5/6 nephrectomy by surgical resection of the upper and lower thirds of the left kidney followed by right nephrectomy 7 days later as described previously [11]. The nephrectomy procedures were carried out through dorsal incisions. The animals assigned to the Sham group were subjected to sham operation. Strict hemostasis and aseptic techniques were assured. The rats were maintained under the above-mentioned conditions for 3 weeks, and were subjected to 70% partial hepatectomy (PHx). Briefly, subcostal incision was performed and the liver was freed from its ligaments. The left lateral and median lobes (equivalent to 70% of the liver) were ligated with 4-0 silk sutures and resected. All surgical procedures were performed under general anesthesia by inhalation of diethyl ether.

*Biochemical assay of blood sample*. Blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (T-bil) were measured by standard laboratory methods.

*Parameters of regeneration*. Three regeneration-associated markers were evaluated. The restitution of the liver weight was determined as the percentage of regenerated liver mass and calculated by the following equation: hepatic regeneration rate (%) $= 100 \times [C-(A-B)]/A$; in which $A$ is the estimated total liver weight at the time of the partial hepatectomy, $B$ is the excised liver weight, and $C$ is the weight of the regenerated liver at the final resection [12]. The mitotic index was determined in paraffin-embedded liver samples stained with hematoxylin and eosin. The mitotic index was expressed as the percentage of mitotic hepatocytes per the total number of hepatocytes in high-power fields (HPF) [12]. Six fields from one specimen were evaluated for six animals in each group. In another experiment, liver samples were stained for proliferating cell-nuclear antigen (PCNA). PCNA is a cell-cycle nuclear protein (molecular weight 36 kD), which is expressed in the late G1 and throughout the S-phase of the mitotic cycle. The amount of PCNA expression correlates with the degree of cell proliferation [13]. PCNA expression was measured using commercially available ZYMED® PCNA STAINING KIT (Zymed, South San Francisco, CA, USA) and performed as recommended in the manufacturer's instruction. The proliferation index of PCNA-stained tissue was determined in HPF. Data were expressed as the percentage of PCNA-stained hepatocytes per total number of hepatocytes.

*Determination of liver mRNA expression by real-time RT-PCR*. The mRNA levels of hepatocyte growth factor (HGF), interleukin (IL)-6, tumor necrosis factor (TNF)-α, c-fos, c-jun, and c-myc in the liver were determined by comparative quantitative real-time RT-PCR using the Mx3000P Real-Time PCR System (Stratagene, La, Jolla, CA, USA). Total RNA was isolated from liver tissues using Qiagen RNeasy mini kit (Qiagen, GmbH, Germany) according to the manufacturer's protocol. cDNA was generated from the total RNA samples using a SuperScript III reverse Trascriptase reagent (Invitrogen, Carlsbag, CA). Each reaction was performed in a 20-μl reaction mixture containing cDNA, 2× PCR Master Mix (Applied Biosystems, Foster City, CA), and each probe and primer set. TaqMan gene expression assays (Applied Biosystems) for HGF, IL-6, TNF-α, c-fos, c-jun, c-myc, and 18S rRNA (endogenous control) were purchased as a probe and primer set (HGF, Rn00566673_m1; IL-6, Rn00561420_m1; TNF-α, Rn01525860_g1; c-fos, Rn00582193_m1; c-jun, Rn00572991_s1; c-myc, Rn00561507_m1; 18S rRNA, Hs99999901_s1). The reaction mixture was denatured for one cycle of 10 min at 95 °C, and incubated for 40 cycles (denaturing for 15 s at 95 °C and annealing and extending for 1 min at 60 °C). All samples were tested in duplicate and average values were used for quantification. Analysis was performed using MxPro TM Software version 2.00 (Stratagene) according to the manufacturer's instructions. The comparative cycle threshold ($C_T$) method ($\Delta\Delta C_T$) was used for quantification of gene expression. The average of the Sham group was set as one-fold induction and other data were adjusted to that baseline.

*Measurement of biliary excretion of indocyanine green*. The biliary excretion of indocyanine green (ICG) were analyzed. Seven days after PHx, a PE-10 catheter was inserted into the common bile duct by laparotomy and the left femoral vein was also cannulated with a PE-50 catheter for the administration of ICG both Sham and CRF groups. After 15 min, when the bile flow became stable, ICG (0.5 mg/kg) was administered via the femoral vein and bile samples were collected every 15 min for 30 min after ICG injection. The bile samples containing ICG were diluted with 0.1 N NaOH after centrifugation and the absorbance at 805 nm was measured.

*Statistical analysis*. There were 6–10 animals in each group. The results were presented as means ± standard error (SE). One-way ANOVA followed by the Student–Newman–Keuls test for multiple comparisons was used to determine the significant differences among the experimental groups. When criteria for parametric testing were violated the appropriate non-parametric (Mann–Whitney $U$-test) test was used. Student's $t$-test was used to compare two groups. A $p$ value less than 0.05 was considered to indicate a significant difference.

## Results

### *Plasma parameters of renal and hepatic functions*

Although plasma AST, ALT, and total bilirubin levels were not significantly different between Sham and CRF rats, BUN and Cr levels were significantly higher in CRF as compared to Sham rats (Table 1). After partial hepatectomy, plasma BUN and Cr levels did not show any significant change from the pre-operative values both in Sham and CRF groups. In contrast, plasma AST, ALT, and total bilirubin levels increased significantly on day 1 and 2 after PHx as compared to pre-operative values both in Sham and CRF groups. However, no significant intergroup dif-

Table 1
Baseline hepatic and renal function

| | Sham ($n = 6$) | CRF ($n = 6$) |
|---|---|---|
| BUN (mg/dl) | 20.23 ± 1.15 | 68.90 ± 5.57* |
| Cr (mg/dl) | 0.24 ± 0.01 | 0.84 ± 0.09* |
| AST (IU/L) | 88.50 ± 3.52 | 95.50 ± 6.70 |
| ALT (IU/L) | 51.50 ± 1.94 | 53.00 ± 4.14 |
| T-bil (mg/dl) | 0.03 ± 0.00 | 0.04 ± 0.00 |

The values are shown as means ± SE. *$p < 0.05$ vs. Sham. Plasma blood urea nitrogen (BUN), creatinine (Cr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (T-bil) revel in sham and experimental chronic renal failure(CRF) rats.