Int J Clin Exp Pathol 2015;8(6):6554-6559
www.ijcep.com /ISSN:1936-2625/IJCEP0007704

Original Article

Different doses of partial liver irradiation promotes hepatic regeneration in rat

Ying Liu[1], Changzheng Shi[2], Meng Cui[2], Zhenhua Yang[1], Danhui Gan[3], Yiming Wang[1]

[1]Department of Oncology, The First Affiliated Hospital of Jinan University, Guangzhou 510630, China; [2]Medical Imaging Center, The First Affiliated Hospital of Jinan University, Guangzhou 510630, China; [3]Department of Pathology, The First Affiliated Hospital of Jinan University, Guangzhou 510630, China

Received March 8, 2015; Accepted April 21, 2015; Epub June 1, 2015; Published June 15, 2015

Abstract: The aim of this study is to investigate whether partial liver irradiation promotes hepatic regeneration in rat. Left-half liver of rat was irradiated to 10 Gy, and the Right-half to 0, 5, 10 and 15 Gy, respectively. Then, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels were evaluated on 0 day, 15-day, 30-day, 45-day and 60-day after liver irradiation. Next, the serum HGF, NF-κB and TGF-β1 levels were also analyzed on 60-day after liver irradiation. Lastly, the cyclinD1 protein expression was appraised by western blots on 60-day after liver irradiation. ALT, AST and ALP levels were reduced compared with that of controls. The serum HGF, NF-κB and TGF-β1 levels, and the cyclinD1 protein expression in liver irradiation group were increased compared with that of controls group. However, hepatic regeneration of higher dose-irradiated cirrhotic liver was triggered a more enhanced regeneration, compared with that of higher doses group. In summary, these results suggest that different doses of partial liver irradiation promotes hepatic regeneration in rat.

Keywords: Liver irradiation, hepatic regeneration, HGF, NF-κB, TGF-β1

Introduction

Radiation-induced liver injury is one of the most deadly complications in the radiation therapy of primary liver cancer, and there is no effective treatment now [1]. The majority of radiation-induced liver injury patients die of liver failure in the short term, but the most effective prevention of radiation hepatitis the method is to limit the average dose of total hepatic irradiation, and determine radiation treatment planning of primary liver cancer combined with V20, V30, V40 and V50, but the provisions of the current domestic and international data show that these data are not entirely consistent, so how to prevent radiation-induced liver damage becomes a hotspot and key issue of liver radiation therapy research [2, 3]. Unlike any other normal organ of the body, the liver of an adult is almost inactive, of which the liver cell division index is less than 1/1000. However, the liver has a powerful regenerative potential; when the liver is damaged, it can be proliferated and regenerated, and hepatic injury factor may be cut off by surgery or chemicals of liver toxicity [4]. Currently, there has been very in-depth study in the liver regeneration after partial hepatectomy. Liver regeneration does not refer to the regeneration of the part that is cut off, but the one of the remnant liver to replace the loss of liver volume and liver function [5].

The study of liver regeneration after partial hepatectomy has been very thorough, but there are few studies on the liver regeneration after the liver injuries induced by different doses of irradiation [6]. Whether radiation therapy as a physical injury to the liver can stimulate the regeneration of exposed liver, and the number of doses received by liver cells to lose the ability to regenerate are not certain [7]. Due to the lack of research, it cannot be judged which has greater influence liver regeneration, small volume of normal liver patient receiving high dose or large volume of normal liver patient receiving small dose [8, 9]. Therefore, the study of liver regeneration capacity of the liver after different doses of irradiation has important clinical sig-

nificance for the clinical program decision of liver cancer radiotherapy. Therefore, this work set out first to explore whether different doses of partial liver irradiation promotes hepatic regeneration in rat.

Materials and methods

Animals

Pathogen free male Wistar rats obtained from the Laboratory Animal Division of the First Affiliated Hospital of Jinan University, weighing 250 ± 25 g, were maintained at a constant temperature in a 12/12-hour light/dark cycle. This research was approved in accordance with Jinan University Guidelines and Regulations on the Use and Care of Lab Animals. All rats were given free access to diets and water ad libitum.

Liver irradiation

Rats were anesthetized with intraperitoneal injection (i.p.) of 35 mg/kg pentobarbital and left-half liver were irradiated in prone position with 6 MV-X ray at a dose rate of 10 Gy/min. Liver irradiation field was marked using superior margin on diaphragm dome, inferior margin at costal arch on each side, and the lateral margin covering both abdominal edges, while the rest of rat was protected by lead shield. Vertebral was marked for separating line. The dose rate of liver irradiation was calculated through liver middle plane upon the measurements by ion chamber in a phantom.

Experiment design

All control groups (Con, n = 6) were irradiated 0 Gy/min. All liver irradiation rats were randomly allocated into five groups: only right-half liver was irradiated to single doses of 0, 5, 10 and 15 Gy/min, respectively. Each liver irradiation group contained 30 rats.

Sample collection

After animals sacrificed, blood samples and liver tissues were collected immediately. Two pieces of liver tissues, 0.5 cm × 0.5 cm × 0.5 cm each, were collected from both left-and right-half livers. These samples were saved at -80°C for further study.

Evaluate hepatic injury, HGF, NF-κB and TGF-β1

The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels were evaluated by HITACHI biochemical analyzer and determined using standard kits (RapidBio Lab, CA, USA). The serum hepatic growth factor (HGF), NF-κB and transforming growth factor beta-1 (TGF-β1) levels were evaluated by ELISA kits (RapidBio Lab, CA, USA).

Western blots of cyclinD1

Right-half livers were homogenized and nuclear proteins were extracted with BCA assay according to the manufacturer's instructions (Roche, Basel, Switzerland). Equal amount of protein were electrophoresed with 12% SDS-polyacrylamide gels and transferred into PVDF by electromembrane transfer at 4°C for 2 hours. The membranes were blocked with TBS-0.05% Tween 20 (TBST) containing 5% non-fat milk to block nonspecific binding sites for 2 h at room temperature. Then, the membranes were incubated with anti-cyclinD1 (Pierce Biotechnology, Rockford, IL, USA), and anti-β-actin (1:1000, Cell Signaling Technology, Boston, MA) overnight at 4°C. The membranes were washed with TBST solution thrice and incubated with anti-mouse IgG (1:1000, Beijing Applygen Technologies, Inc., Beijing, China) conjugated for 2 h at room temperature. All membranes were visualized by using chemiluminescence substrate system (Verity Software House Inc., Topsham, ME).

Statistical analysis

The data were performed with SPSS 17.0 software. Data were expressed as mean ± standard deviation (SD) and analyzed by using Student two-tailed t test. *P* value of < 0.05 was considered significant.

Results

Different doses of partial liver irradiation induced liver-injury ALT, AST and ALP levels

To investigate the possible effects of partial liver irradiation (0, 5, 10 and 15 Gy/min) on ALT, AST and ALP levels, the serum ALT, AST and ALP levels of rats were analyzed using standard kits. For rats with right-half liver irradiation, partial liver irradiation significantly induced liver-injury the serum ALT, AST and ALP levels, compared with control group (**Figure 1**).

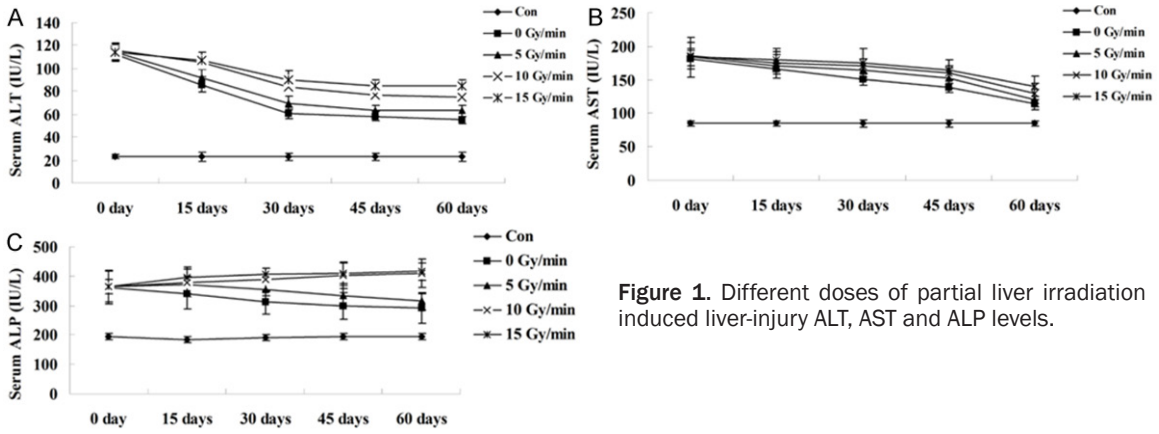

Figure 1. Different doses of partial liver irradiation induced liver-injury ALT, AST and ALP levels.

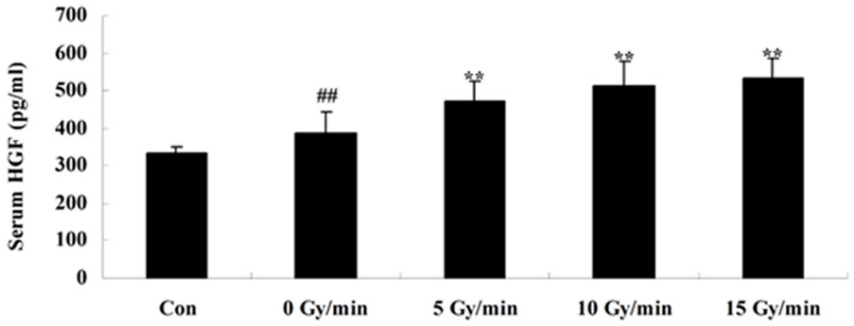

Figure 2. Different doses of partial liver irradiation induced liver-injury HGF level. ##*P*<0.01 versus the control group, ***P*<0.01 versus 0 Gy/min partial liver irradiation group; Con, control group; 0 Gy/min, 0 Gy/min partial liver irradiation group; 5 Gy/min, 5 Gy/min partial liver irradiation group; 10 Gy/min, 10 Gy/min partial liver irradiation group; 15 Gy/min, 15 Gy/min partial liver irradiation group.

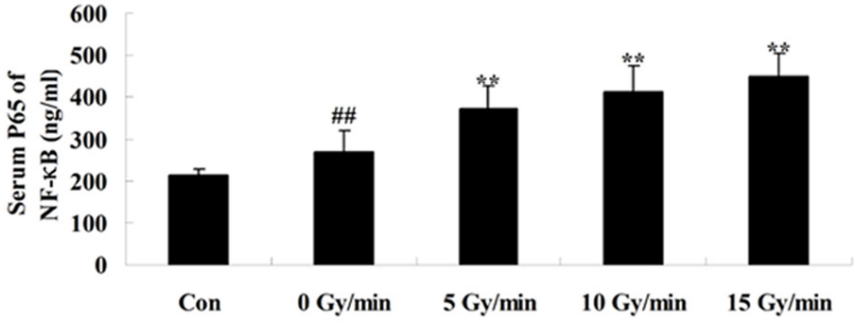

Figure 3. Different doses of partial liver irradiation induced liver-injury NF-κB level. ##*P*<0.01 versus the control group, ***P*<0.01 versus 0 Gy/min partial liver irradiation group; Con, control group; 0 Gy/min, 0 Gy/min partial liver irradiation group; 5 Gy/min, 5 Gy/min partial liver irradiation group; 10 Gy/min, 10 Gy/min partial liver irradiation group; 15 Gy/min, 15 Gy/min partial liver irradiation group.

Different doses of partial liver irradiation induced liver-injury HGF level

To assess that the possible effects of partial liver irradiation (0, 5, 10 and 15 Gy/min) on HGF level, the serum HGF level of rats was analyzed using ELISA kits. For rats with right-half liver irradiation, liver-injury the serum HGF level was effectively increased by partial liver irradiation (0 Gy/min), compared with control group (**Figure 2**). However, partial liver irradiation (5, 10 and 15 Gy/min) effectively enhanced liver-injury the serum HGF level, compared with partial liver irradiation (0 Gy/min) group (**Figure 2**).

Different doses of partial liver irradiation induced liver-injury NF-κB level

To appraise that the possible effects of partial liver irradiation (0, 5, 10 and 15 Gy/min) on NF-κB level, the serum NF-κB level of rats was analyzed using ELISA kits. For rats with right-half liver irradiation, liver-injury the serum NF-κB level was effectively increased by partial liver irradiation (0 Gy/min), compared with control group (**Figure 3**). However, partial liver irradiation (5, 10 and 15 Gy/min) effectively enhanced

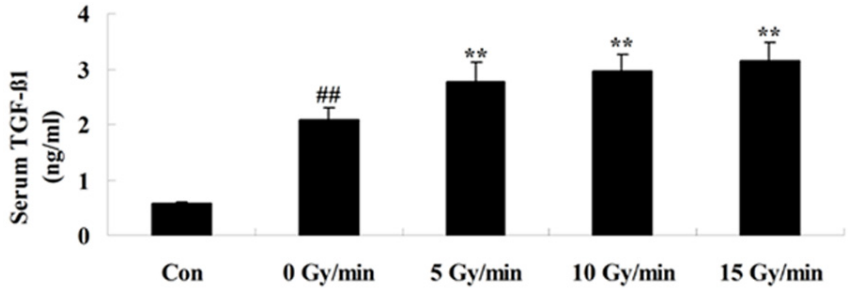

Figure 4. Different doses of partial liver irradiation induced liver-injury TGF-β1 level. ##P<0.01 versus the control group, **P<0.01 versus 0 Gy/min partial liver irradiation group; Con, control group; 0 Gy/min, 0 Gy/min partial liver irradiation group; 5 Gy/min, 5 Gy/min partial liver irradiation group; 10 Gy/min, 10 Gy/min partial liver irradiation group; 15 Gy/min, 15 Gy/min partial liver irradiation group.

liver-injury the serum NF-κB level, compared with partial liver irradiation (0 Gy/min) group (**Figure 3**).

Different doses of partial liver irradiation induced liver-injury TGF-β1 level

To appraise that the possible effects of partial liver irradiation (0, 5, 10 and 15 Gy/min) on TGF-β1 level, the serum TGF-β1 level of rats was analyzed using ELISA kits. For rats with right-half liver irradiation, liver-injury the serum TGF-β1 level was effectively increased by partial liver irradiation (0 Gy/min), compared with control group (**Figure 4**). However, partial liver irradiation (5, 10 and 15 Gy/min) effectively enhanced liver-injury the serum TGF-β1 level, compared with partial liver irradiation (0 Gy/min) group (**Figure 4**).

Different doses of partial liver irradiation induced liver-injury cyclinD1 protein expression level

To evaluate that the possible effects of partial liver irradiation (0, 5, 10 and 15 Gy/min) on TGF-β1 level, the cyclinD1 protein expression level of rats was analyzed using western blots. For rats with right-half liver irradiation, liver-injury the cyclinD1 protein expression level was effectively increased by partial liver irradiation (0 Gy/min), compared with control group (**Figure 5A, 5B**). However, partial liver irradiation (5, 10 and 15 Gy/min) effectively enhanced liver-injury the cyclinD1 protein expression level, compared with partial liver irradiation (0 Gy/min) group (**Figure 5A**, **5B**).

Discussion

Liver regeneration after liver resection is a very complex process, not only the regeneration of a single organ, liver, but also including systematic stress reactions of the body caused by hepatectomy [10]. Therefore, future research should focus on the overall situation, not restricted in one organ, liver. In-depth study on liver regeneration after hepatectomy is helpful for the liver function recovery of patients with acute or chronic liver failure and the reduction of overall health care costs and mortality of patients with liver failure [11]. At present, China ranks first in the world in patients with hepatitis B, with more than 100 million hepatitis B virus carriers. Every year, the number of the patients with liver resection for a variety of liver diseases is also increasing, so the liver regeneration study has great significance for the health of people in our country [12, 13]. In this study, different doses of liver irradiation could reduce the serum ALT, AST and ALP levels, and aggrandized liver-injury the serum HGF level. But, we found that partial liver irradiation (10 and 15 Gy/min) slightly elevated the serum AST level of rats. This result should be attention, and hinted that high doses of partial liver irradiation increased the serum AST level. Liver has strong regeneration ability; liver regeneration is a special, proliferative reaction of multiple effector cells the expression of a variety of regulatory factors, and the complex cell proliferation process that involves synchronous or sequential activation and interaction of multiple pathways [14]. Resting hepatocytes must enter into GI phase from the G0 phase in order to obtain proliferativity, thus into the liver regeneration start-up phase [15]. Cytokines (TNF-α, IL-6) and transcription factors (NF-κB) are the most important signal regulatory molecules for the start phase of liver regeneration, which form an important signal transduction pathway for the start-up phase of liver regeneration: TNF-α → TNFR-I → NF-Kb → IL-6 → STAT-3 [16]. In our study, different doses of liver irradiation effectively increased liver-injury the serum NF-κB level. Our results explained that different doses of liver irradiation could augment the serum NF-κB level and promote hepatic regeneration in rat through NF-κB signal path. Transforming growth factors-β (TGF-β)

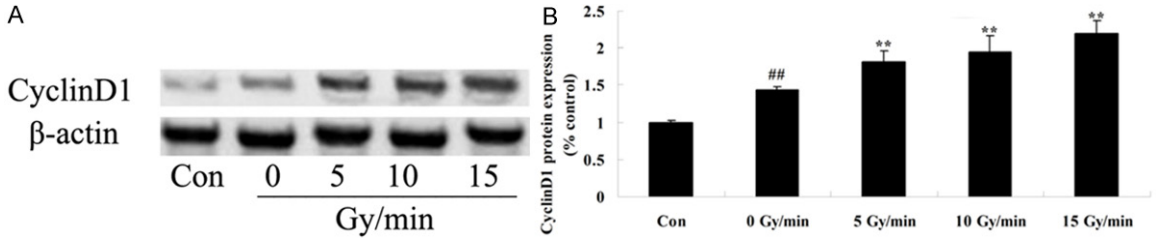

Figure 5. Different doses of partial liver irradiation induced liver-injury CyclinD1 protein expression level. Different doses of partial liver irradiation induced liver-injury CyclinD1 protein expression level using Western blot analysis, statistical analysis of CyclinD1 protein expression in rat. ##P<0.01 versus the control group, **P<0.01 versus 0 Gy/min partial liver irradiation group; Con, control group; 0 Gy/min, 0 Gy/min partial liver irradiation group; 5 Gy/min, 5 Gy/min partial liver irradiation group; 10 Gy/min, 10 Gy/min partial liver irradiation group; 15 Gy/min, 15 Gy/min partial liver irradiation group.

is a class of cytokines with a variety of chemical and physical functions, playing an important role in the development of liver fibrosis, which may regulate the cell proliferation and differentiation, promote cell synthesis and secretion of extracellular matrix, reduce the degradation of extracellular matrix, and play an important role in angiogenesis, physiological and pathological embryonic development, tissue repair, immune regulation, tumorigenesis and other processes in the organism, and its high expression can promote the pathological effect of tissue fibrosis, parallel with the degree of liver fibrosis in pathology [17-19]. Studies have shown that TGF-β1 is related to the termination process of liver regeneration. We found that different doses of liver irradiation induced the serum TGF-β1 level in rats. 10-14 d can fully recover missing liver tissue after 70% partial hepatectomy of rat liver. In the process, a variety of cells are proliferated rapidly and orderly, most of the remaining liver cells reach a peak in DNA synthesis after 24 hours, and a small number of liver cells have secondary DNA synthesis [20]. CyclinD1 is a rate-limiting factor controlling the G1 phase progression, considered as the symbol that the cells enter into the G1 phase of the cell cycle [21]. CyclinD1 protein expression in hepatocyte nucleus reaches the peak 24 hours after partial resection in rat liver [22]. The study of liver regeneration in rat shows the dynamic change of cyclinD1 is closely related to liver regeneration. This research shows that different doses of liver irradiation enhanced the cyclinD1 protein expression level in rats.

In summary, different doses of partial liver irradiation (5, 10 and 15 Gy/min) promotes hepatic regeneration through activating NF-κB, TGF-β1 and cyclinD1 in rat. This work supports further investigation to expound the detailed mechanism of partial liver irradiation on hepatic regeneration in rat.

Acknowledgements

The study was supported by Guangdong Province Science and technology plan fund (NO. 2012B031800156).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yiming Wang, Department of Oncology, The First Affiliated Hospital, Jinan University, No. 613, Huangpu West Rd, Guangzhou 510630, China. Tel: 86-020-38688888; Fax: 86-020-38688888; E-mail: Yimingwangna@163.com

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