行政院國家科學委員會補助專題研究計畫成果報告

大鼠肝臟部分切除後，免疫反應與肝細胞再生相關之研究

The study of immune response and liver regeneration after partial hepatectomy in rats

計畫類別：☑個別型計畫　□整合型計畫

計畫編號：NSC89－2314－B－002－212－

執行期間：88 年 08 月 01 日至 89 年 07 月 31 日

計畫主持人：賴鴻緒

共同主持人：

本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

執行單位：國立台灣大學醫學院外科

中　華　民　國　89 年　10 月　09 日

行政院國家科學委員會專題研究計畫成果報告

大鼠肝臟部分切除後，免疫反應與肝細胞再生相關之研究

The study of immune response and liver regeneration after partial hepatectomy in rats

計畫編號：NSC 88-2314-B-002-212
執行期限：88 年 8 月 1 日至 89 年 7 月 31 日
主持人：賴鴻緒　　台大醫學院　外科

中文摘要

肝臟損傷後，肝細胞有再生能力，是醫學研究公認的事實，研究受傷後肝細胞再生之機轉與引因，藉以促進肝細胞再生之加速及肝功能之恢復，亦成為醫學界最重要的議題之一。許多報告指出，肝臟部份切除後，局部或全身性免疫系統會有變化，諸如肝臟內之 extrathymic T cell，lymphokine activated killer cells 均被發現有關連。Sato 等人證實了肝內 extrathymic T cells 會被活化，而 Hirano 等人則實驗顯示肝內淋巴球的變遷有別於血液淋巴球。吾人以老鼠做動物實驗，發現切肝後，血液 T 型淋巴球次群，包括 T3、T4、T8、T-IL2R、NK 等，均明顯增加，血清 γ-IFN 及剩餘肝內 NK 細胞均明顯上升，而血清及尿中 neopterin 之濃度亦大大提高。Tanaka 等人認為 lymphokine-activated killer cell 可抑制肝細胞再生，Ohnishi 等人亦證實了 γ-IFN 的明顯上升亦有關，TNF、TGF 及生長激素也被提到可能有關。本計畫以重約 200 公克之 Wistar 雄性大鼠作研究，分為實驗組及對照組，分別施行百分之七十部份肝臟切除術、右腎切除術、及剖腹探查等手術，在術後第 1、2、3、5、7 及 10 天犧牲取樣，測定(1)血清各種 interleukin (IL) 之濃度，包括 IL-1α、IL-1β、IL-2、IL-4、IL-6 及 IL-8 等(2)血清其他淋巴激素的濃度，包括 TGF-β1、TNF-α、TNF-RI、TNF-RII 及 Insulin-like GF 等；(3)檢視剩餘肝臟組織學變化、細胞分裂情形，及(4)測量血清肝功能之變化。結果發現：(1)IL-1 α，IL-2，IL-6 β 等血清淋巴激素，在術後 5 至 7 天均明顯上升；(2)血清 TNF-α 濃度在術後 5 至 7 天，亦有 8 至 10 倍之升高；(3)肝臟切片，於術後 48 至 72 小時，可在光學顯微鏡下看到許多不同期之有絲分裂現象；(4)血清肝功能指標於術後 6 至 48 小時明顯變差，但 72 小時即逐漸恢復。本實驗證實，肝臟部分切除後，免疫反應與肝細胞再生息息相關，但各種淋巴激素中，有不同反應，其機轉仍需進一步研究。

關鍵詞：肝臟部份切除，肝細胞再生，免疫反應，淋巴激素

ABSTRACT

The regenerative ability of liver cell after liver injury was recongnized by almost all medical centers. How to stimulate liver cell regeneration after liver injury should be also one of the most important aspects. Partial hepatectomy may raise the possibility of changes in local or systemic immune response. Several phenomena such as alterations of extrathymic T cells in the liver, lymphokine-activated killer cells were observed in animal experiments after partial hepatectomy. Sato et al proved that extrathymic T cells in the liver are activated, yet the intrathymic pathway is suppressed. Hirano et al reported that intrahepatic lymphocytes differ from peripheral blood lymphocytes in response to partial hepatectomy. Acute phase response after

partial hepatectomy was proved to be an indicator of cytokine release. Our previous studies also found that all T-lymphocyte subpopulations in peripheral blood, serum γ -IFN, peripheral blood NK cells and the absolute number of NK cells in the remnant liver of hepatectomized rats increased markedly on the fifth and seventh posthepatectomized day. Serum and urinary neopterin concentrations can be used as markers of activated cell-mediated immunity in rats after partial hepatectomy. Tanaka et al proved that lymphokine-activated killer cells can inhibit liver regeneration. Ohnishi et al proved that there was a marked rise of the production of gamma-interferon (γ -IFN), which is known to activate the natural killer (NK) cells. However, there is still a lot of controversy in the relationship between immune response and liver regeneration. Male Wistar rats around 200g were used as subject in this study. Partial hepatectomy (about seventy percent hepatectomy) was performed on experimental group rats. They were sacrificed at 1,2,3,5,7 and 10 days after hepatectomy. We will measure (1) serum concentrations of inflamatory interleukins (IL) including IL-1α, IL-1β, Il-2, IL-4, IL-6 and IL-8; (2) other serum lymphokines, including TGF-β1, TNF-α, TNF-RI, TNF-RII and Insulin-like GF; (3) histological changes of the remnant liver (including mitosis and regeneration condition); (4) serum liver function tests. The results showed that: (1) Serum levels of IL-1 α, IL-2, IL-6 increased markedly on 5^{th} to 7^{th} posthepatectomized day ; (2)Serum TNF-α level increased to 8 to10 times 5 to 7 days after operation ; (3) Many mitosis pictures could be seen in histopathological liver section ; (4) Liver function became deterious at 6 to 48h, then recovered gradually 72h after hepatectomy. We concluded that immune response is marked during the period of liver regeneration. However, the response is different between different lymphokines. Further study for the mechanism is needed.

Key words: partial hepatectomy, liver regeneration, immune response, lymphokines

INTRODUCTION

Liver is a core organ in our body. Although it contains good regeneration ability, hepatic failure still can occur after massive hepatic injury or hepatectomy. The capacity of the mammalian liver to regenerate in response to numerous stimuli has been well documented. Many studies have focused on factors which can promote or regulate liver regeneration. Several factors, such as hormones, growth factors, nutritional components, and pharmacological agents, have been demonstrated to directly or indirectly affect liver regeneration. Much controversy continues on the initiation, regulation, metabolic changes, and termination of liver regeneration after partial hepatectomy that may well initiate proliferation of the remaining hepatocytes.

Partial hepatectomy may raise the possibility of changes in local or systemic immunological response. Several phenomena such as alterations of extrathymic T cells in the liver, lymphokine activated killer cells were observed in animal experiments after partial hepatectomy. Sato et al proved that extrathymic T cells in the liver are activated, yet the intrathymic pathway is suppressed. Hirano et al reported that intrahepatic lymphocytes differ from peripheral blood lymphocytes in response to partial hepatectomy. Acute phase response after partial hepatectomy was proved to be an indicator of cytokine release. Koga et al also reported that interleukin 1 and 6 can induce hepatocyte mitosis. However, Tanaka et al proved that lymphokine-activated killer cells can inhibit liver regeneration following extended hepatectomy. Some other studies also came to the conclusion that immunologic response can regulate or inhibit liver regeneration after partial hepatectomy. Ohnishi et al proved that there was a marked rise of the production of gamma-interferon (γ -IFN),

which is known to activate the natural killer (NK) cells, by the splenic mononuclear cells in mice after 70% partial hepatectomy. They also suggested that the NK cells which activated by γ -IFN may be involved in killing the regenarating liver cells. Vujanovic et al studied the phenotype and function of liver-resident NK cells after 70% partial hepatectomy, and suggested that liver-resident NK cells may be involved in the regulation of the extent of liver regeneration. Tumor necrosis factor, transforming growth factor and other growth factors were also mentioned. The relationship between immune response and liver regeneration has become an important focus for study. However, there is still a lot of controversy.

MATERIALS AND METHODS

Experimental Protocol

Male Wistar rats (purchased from Charles River, Osaka, Japan) weighing approximately 200 g were used as subjects. All the rats were randomly assigned to four different groups.Group 1(N=60), Partial hepatectomy (H). Group 2(N=60), right-side nephrectomy (N). Group 3(N=60), sham operation with laparotomy only (S). Group 4(N=60), control group with no operation (C). Each groups are divided into six subgroups (N=10 in each): sacrificed on 1、2、3、5、7、and 10 days after operation.

Surgical Procedures

All rats are anesthetized by intraperitoneal pentobarbital (40mg/Kg) injection. A midline laparotomy was performed. Partial hepatectomy was then carried out by means of aseptic extirpation of the median and left lateral lobes, according to the procedure of Higgins and Anderson . The removed liver sample was immediately weighed. Ligation of right-side renal artery and vein, followed by nephrectomy was performed on N group rats. Laparotomies with manipulation of liver were done in the sham operated rats. All of the surgeries were performed only between 8 am and 11 am to reduce the influence of diurnal variation. Ten rats from each group were sacrificed at 1, 2, 3, 5, 7 and 10 days after the operations, and the livers were removed immediately. A small piece of liver was cut and put into formalin solution for histological observation. Blood sample was also aspirated at the same time for biochemical and immunological studies. All the procedures were performed by the same surgeon to reduce the influence of surgical technical variation.

Sampling and Measurements

Immunological assesements were performed insluding serum interleukins (IL) IL-1α, IL-1β, IL-2, IL-4, IL-6 and IL-8 by enzyme immune assay (EIA) kits. (#ER-IL1A, Endogen Inc.; #KRC0012, #DRC0022, #KRC0042, #KRC0062, #KRC0080-SB, Biosource Inc.). Serum concentration of transforming growth factor beta-1 (TGF-β1) (#DB100, R & D system)、tumor necrosis factor (TNF)(#KRC3012, Biosource)、tumor necrosis factor receptor (including TNF-RI and TNF-RII) (#KAC-1762, #DAC-1772, Biosource Inc) were measured by EIA kits. Histological examination of remnant liver and serum biochemical indicators of liver function tests were also performed during the early experimental period.

RESULTS

The changes of serum lymphokines were different. The serum level of IL-1 α increased to about two times (18.9 $\pm$4.0 to 40.2 $\pm$6.0 and 37.9 $\pm$5.8 pg/ml) at 5th and 7th posthepatectonized days. Then it returned to preoperative level (Fig, 1). The serum IL-2 level increased somewhat later. It increased from 11.2 $\pm$1.8 pg/ml on day 0 to 22.6 $\pm$3.8 pg/ml on day 5. Then it increased more marbed to 41.1 $\pm$6.4 and 32.6 $\pm$5.8 pg/ml on day 7 and 10 after surgery (Fig 2) . The increase of serum IL-6 levels were shown in Fig 3. The level increased from 52.6 $\pm$8.8 pg/ml on day 0 to 200 $\pm$36, 186 $\pm$31 pg/ml

on day 5 and 7. Then the level increased more to 385 ±62 pg/ml on day 10. The changes of serum TNF-α were shown on Fig 4. It increased from 0.89 ±0.18 pg/ml on day 0 to 6.82 ±0.88 pg/ml on day 5. It increased more up to 8.10 ±1.03 pg/ml on day 7. Then it suddenly returned to preoperative level. As for the serum levels of other lymphokines (including IL-1β, IL-4, IL-8, TGF-β1, TNF-R I, TNF-R II and Insulin-like GF), there were no significant changes could be found in this study.

Observation of the paraffin sections of the liver tissue under a microscope demonstrated that : there were almost no mitoses in the sham operated rat group, and only a few mitoses of liver parenchymal cells could be observed at the 24 hour stage. They increased to around 100 in 50 different fields under 400x high power field at 48 hours postoperatively. We could find no significant difference among all the experimental groups and the control rat group. The number decreased to around one-fourth at the 72-hour stage in all rat groups. The serum parameters of the liver function test revealed a somewhat elevated level of GOT, GPT, and alkaline phosphate within the first 48 hours after partial hepatectomy in all rat groups. No significant changes could be found in the bilirubin, albumin, and globulin levels. There was a more marked elevation of GOT and GPT at 24 hours postoperatively and a greater elevation of alkaline phosphate at 48 hours postoperatively in the LG rat group, than in the control and HG rat groups ($p<0.05$).

DISCUSSION

The phenomenon of liver regeneration is one of the most rapid forms of tissue growth occurring in mammals. It happened in the very early phase and slowed down gradually after partial hepatectomy, as was proven by previous studies here and in many other centers. In recent studies, hepatectomy has been shown to be able to induce not only proliferation of hepatocytes, but also lymphoid tissues. Ono et al reported that lymphokine-activated killer cells induced from spleen of hepatectomized mice were cytotoxic against regenerating liver cells. Tanaka et al made another conclusion that liver regeneration can be regulated by interleukin 2 and its inhibitors: cyclosporine A and FK506. Prostaglandin E2 production during hepatic regeneration downregulates Kupffer cell, as Goss et al also proved. Other studies Kupffer cells and sinusoidal endothelial cells, can induce simultaneously the activation of extrathymic T-cell differentiation and the inactivation of intrathymic T-cell differentiation. Michalopoulos and DeFrances reviewed many studies and suggested that many interleukins and TNF, TGF, Insulin like growth factor may be important in the process of liver regeneration. However, whether these are two independent phenomenon or one follows the other is still unclear.

In this study, we found that the lymphokines have shown different changes in serum levels after partial hepatectomy. The significant changes could only be identified in serum IL-1α, IL-2, IL-6, and TNF-α levels. The changes of serum levels were different in these four lymphokines. The highest serum level was identified on days 5 and 7 in IL-1α, on days 7 and 10 in IL-2, on day 10 in IL-6, and on day 7 in TNF-α. Furthermore, the increased discrepancy of serum levels in each lymphokines were also not the same. It increased to around double in IL-1α, around 3 to 4 times in IL-2, 7 time in IL-6, and more than 8 times in TNF-α. The mechanism was nor clear. It demonstrated that each lymphokines has its own change in the pathway of immune response after partial hepatectomy.

The speed of liver regeneration were different in different animal and different cause of liver injury. In this animal model of 70 % partial hepatectomy in rats. The regeneration rate is very high in the early stage and reach to complete around 10 to 14