

Changes of immune function in patients with liver cirrhosis after splenectomy combined with resection of hepatocellular carcinoma

Zhi-Xin Cao, Xiao-Ping Chen and Zai-De Wu

Wuhan, China

OBJECTIVE: To study the changes of immune function in liver cirrhosis patients after splenectomy combined with resection of hepatocellular carcinoma (HCC).

METHODS: Sixteen patients with HCC associated with liver cirrhosis were divided into two groups: splenectomy combined with hepatectomy (splenectomy group $n = 7$) and hepatectomy (non-splenectomy group, $n = 9$). T lymphocyte subsets such as CD4, CD8, CD4/CD8 and Th lymphocyte cytokines such as interferon γ (IFN- γ), IL-2, IL-10 in 7 ml peripheral venous blood before operation and 2 months after operation were examined and compared between the two groups.

RESULTS: There was no significant difference in pre-operative CD4, CD8, CD4/CD8, IL-2, IFN- γ , IL-10 levels in the two groups. Two months after operation, the levels of CD4 ($38.2\% \pm 3.7\%$), CD4/CD8 (1.7 ± 0.3), IFN- γ (104.4 ± 14.9 pg/ml), IL-2 (98.6 ± 18.6 pg/ml) were increased and those of CD8 (23.7 ± 3.7 pg/ml), IL-10 (55.5 ± 11.2 pg/ml) levels were decreased in the splenectomy group. The levels of CD4 ($32.5\% \pm 4.0\%$), CD4/CD8 (1.1 ± 0.1), IFN- γ (70.5 ± 12.6 pg/ml), IL-2 (80.9 ± 13.5 pg/ml) in the non-splenectomy group were much lower than those in the splenectomy group, but the levels of CD8 ($29.4\% \pm 4.0\%$), IL-10 (89.4 ± 10.0 pg/ml) in the non-splenectomy group were significantly higher than those in the splenectomy group ($P < 0.05$).

CONCLUSIONS: Splenectomy combined with hepatectomy for HCC patients associated with liver cirrhosis does not decrease but promote the recovery of T lymphocyte subsets and Th1/Th2 cytokines from imbalance and improve anti-tumor immune function of the patients.

(*HBPD Int* 2003; 2: 562–565)

Key words: hepatocellular carcinoma; liver cirrhosis; splenectomy; immunity

Introduction

Hepatocellular carcinoma (HCC) is highly prevalent in China, with a yearly death rate of 110 000 or about 45% of deaths from this disease in the world. Of all HCC patients, 85%–90%

were associated with liver cirrhosis, hypersplenic thrombocytopenia and others, which influence its treatment and prognosis. To the present, the study whether splenectomy is performed on these patients has been rarely reported. We studied the changes of immune function in liver cirrhosis patients after splenectomy combined with resection of HCC.

Methods

Patients and samples

HCC patients with liver cirrhosis, hypersplenism (defined as \geq II grade splenomegaly, severe esophageal varices, platelet count $\leq 100 \times 10^9/L$,

From the Hepatic Surgery Center, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China (Cao ZX, Chen XP and Wu ZD)

Correspondence: Zhi-Xin Cao, MD, Hepatic Surgery Center, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China (Tel: 86-27-83662599; Email: czxtj@hotmail.com)

white blood cell $\leq 4 \times 10^9/L$) in our department were selected and divided into two groups: splenectomy combined with hepatectomy (splenectomy group, $n = 7$) and hepatectomy (non-splenectomy group, $n = 9$). Their diagnosis were definite. Before operation, all patients were not treated by radiation therapy, chemotherapy, and immune therapy. In order to determine T lymphocyte subsets such as CD4, CD8, CD4/CD8 and Th lymphocyte cytokines such as interferon γ (IFN- γ), IL-2, IL-10, 7 ml peripheral venous blood was taken from the two groups before and 2 months after operation.

Detection of immune function

T lymphocyte subsets such as CD4, CD8, CD4/CD8 were determined by flow cytometry (FCM), and the monoclonal antibodies of CD4 and CD8 (labelled CD4-FTTC, CD8-PE) were purchased from Coulter Corporation, USA. FCM was performed with a wavelength of 488 nm, power 300 mW, HP-300 Consort 30 (BD Corporation, USA) was utilized to process the data. Th lymphocyte cytokines such as IFN- γ , IL-2, IL-10 were examined by ELISA. ELISA kits were purchased from Dialone Corporation, USA.

Statistical analysis

Statistical analysis was performed using Student's t test. A $P < 0.05$ was considered signifi-

cant.

Results

The changes of T lymphocyte subsets before or after operation in the two groups are shown in Table 1. Before operation, no significant difference was observed in T lymphocyte subsets such as CD4, CD8, CD4/CD8 between the two groups. After operation, T lymphocyte subsets such as CD4, CD4/CD8 were increased and CD8 was decreased in the two groups, but the changes in the splenectomy group was more obvious than those in the non-splenectomy group. Two months after operation, the subsets such as CD4, CD4/CD8 were higher and CD8 was lower in the splenectomy group than in the non-splenectomy group ($P < 0.05$).

The changes of Th lymphocyte cytokines such as IFN- γ , IL-2, IL-10 levels before and after operation in the two groups are shown in Table 2. Before operation, no significant difference was seen in the levels of Th lymphocyte cytokines such as IFN- γ , IL-2, IL-10 between the two groups. After operation, the levels of IFN- γ , IL-2 were increased and the levels of IL-10 decreased in the two groups. The changes in the splenectomy group, however, were more obvious than those in the non-splenectomy group. Two months after operation, the levels of IFN- γ , IL-2 were higher and the le-

Table 1. Changes of T lymphocyte subsets before or after operation in the two groups ($\bar{x} \pm s$, %)

T lymphocyte subsets	Before operation		After operation	
	Splenectomy ($n = 7$)	Non-splenectomy ($n = 9$)	Splenectomy ($n = 7$)	Non-splenectomy ($n = 9$)
CD4	33.5 \pm 4.5	31.9 \pm 8.3	38.2 \pm 3.7*	32.5 \pm 4.0
CD8	32.5 \pm 4.5	32.9 \pm 6.0	23.7 \pm 3.7*	29.4 \pm 4.0
CD4/CD8	1.1 \pm 0.2	1.0 \pm 0.5	1.7 \pm 0.3**	1.1 \pm 0.1

Compared between the two groups after operation: * $P < 0.05$; ** $P < 0.01$.

Table 2. Changes of levels of Th lymphocyte cytokines before and after operation in the two groups ($\bar{x} \pm s$, pg/ml)

Th lymphocyte cytokines	Before operation		After operation	
	Splenectomy ($n = 7$)	Non-splenectomy ($n = 9$)	Splenectomy ($n = 7$)	Non-splenectomy ($n = 9$)
IL-2	79.7 \pm 20.8	72.7 \pm 20.8	98.6 \pm 18.6*	80.9 \pm 13.5
IFN- γ	91.9 \pm 37.3	79.2 \pm 12.6	104.4 \pm 14.9*	70.5 \pm 12.6
IL-10	73.8 \pm 38.5	79.8 \pm 15.3	55.5 \pm 11.2*	89.4 \pm 10.0

* : Compared between the two groups after operation, $P < 0.05$.

vels of IL-10 were lower in the splenectomy group than those in the non-splenectomy ($P < 0.05$).

Discussion

T lymphocyte cell immunity is mainly cell immunity in tumor immunity. CD4 T lymphocyte cells regulate anti-tumor immunity by producing lymphocyte cytokines, but CD8 T lymphocyte cells produce immunosuppressive cytokines and mediate immunosuppression. Thus the detection of T lymphocyte subsets such as CD4, CD8, CD4/CD8 may represent patients cellular immune function. Th lymphocyte cytokines have been found to express two distinct cytokine patterns. Th1 cells, which produce IL-2, IFN- γ and promote cell mediate immune responses, play an important role in anti-tumor immunity. Th2 cells produce IL-4, IL-10 and inhibit Th1 cytokines release and promote immunoglobulin production. To have good anti-tumor immune response, Th1 cytokine expression should predominate.

Jasnis et al^[1] transplanted S13M3 breast cancer to BACB/C mice and found supernatant medium of splenic cell with big size tumor from tumor-bearing mice had much more soluble immunosuppressive factor than those with small size tumor. After tumor resection, the effect of promoting tumor growth still persisted for a long time. It was reported that radical operation 2 (R2 +, non-splenectomy) and radical operation 3 (R3, splenectomy) in patients with advanced gastric cancer 2–4 years after the operations showed no significant difference in immune function of the patients.^[2] Other experiment^[3] showed a lot of suppressive macrophages accumulated in the spleen from tumor-bearing mice altered the structure of T cell receptor (TCR-CD3) and inhibited T cell immune function. We found the predominance of Th2 type cytokines in splenic venous blood of HCC patients associated with liver cirrhosis.^[4]

In our study, no significant difference was observed in preoperative T lymphocyte subsets such as CD4, CD8, CD4/CD8 and levels of Th lymphocyte cytokines such as IFN- γ , IL-2, IL-10 between the two groups. Two months after operation, how-

ever, T lymphocyte subsets such as CD4, CD4/CD8 and the levels of Th1 lymphocyte cytokines such as IFN- γ , IL-2 were higher, but the T lymphocyte subsets CD8, Th2 lymphocyte cytokines IL-10 levels were lower in the splenectomy group than in the non-splenectomy group, indicating the spleen of HCC patients associated with liver cirrhosis inhibited the immune function. After resection of HCC, immunosuppression retained. If splenectomy was performed, immunosuppression decreased gradually. Therefore, splenectomy combined with resection of hepatocellular carcinoma does not decrease but improve the recovery of T lymphocyte subsets and Th lymphocyte cytokines from imbalance, which is ultimately helpful for the strengthening of anti-tumor immune function. In HCC patients associated with liver cirrhosis, we also found the lower white blood cells and platelet count increased immediately after splenectomy combined with resection of HCC, which is a promising condition for chemotherapy after operation. Splenectomy combined with resection of HCC reduces the serum bilirubin concentration and somewhat improves liver function too.^[5] This operation also promotes hepatic and regeneration,^[6] decreases portal vein pressure and the rate of variceal bleeding of the esophagus to some extent. Moreover, the low incidence of postoperative complications has indicated that splenectomy combined with resection of HCC is a safe procedure. According to the report,^[7] the overall 5-year actuarial and disease-free survival rate is 66.7% after splenectomy combined with resection of HCC in patients associated with liver cirrhosis. It is higher than that after hepatectomy.^[7] We conclude that splenectomy combined with resection of HCC in patients associated with liver cirrhosis is justified as the benefits of concomitant splenectomy by far surpass the adverse effects.

Competing interest

The author or authors do not choose to response to the statements listed in Instructions for Authors.

References

- 1 Jasnis MA, Eljan AM, Oisgold-Daga S. Regulation of

- tumor growth by soluble spleen factor; effect of tumor resection. *J Surg Oncol* 1987;35:139-146.
- 2 Zhu ZG, Lin YZ, Yin HR, et al. The study on splenic immunological condition and surgical treatment in advanced gastric cancer. *Natl Med J China* 1992;76:330-333.
 - 3 Aoe T, Okamoto Y, Saito T. Activated macrophages induce structural abnormalities of the T cell receptor-CD3 complex. *J Exp Med* 1995;181:1881-1886.
 - 4 Cao ZX, Chen XP, Wu ZD. A study on Th1/Th2 cytokines immunological condition in the patients with primary hepatocellular carcinoma combined with liver cirrhosis. *Chin J Exp Surg* 2001;18:518-519.
 - 5 Cao ZX, Chen XP, Wu ZD. Splenectomy combined with hepatectomy in cirrhotic patients suffering from primary liver cancer. *Chin J Gen Surg* 2002;17:328-329.
 - 6 Tomikawa M, Hashizume M, Higashi H, et al. The role of the spleen, platelets and plasma hepatocyte growth factor activity in rats. *J Am Coll Surgeons* 1996;182:12-16.
 - 7 Lin MC, Wu CC, HO WL, et al. Concomitant splenectomy for hypersplenic thrombocytopenia in hepatic resection for hepatocellular carcinoma. *Hepato-Gastroenterology* 1999;46:630-634.

Received March 3, 2003

Accepted after revision March 24, 2003

In most of mankind gratitude is merely a secret hope for greater favours.

—Duc de la Rochefoucauld