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He Jie Tang in the treatment of chronic hepatitis B patients

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Abstract

AIM: To explore the effect of He Jie Tang (decoction for medication) on serum levels of T lymphocyte subsets, NK cell activity and cytokines in chronic hepatitis B patients.

METHODS: Eighty-five patients with chronic hepatitis B were divided randomly into two groups. Fifty patients in group I were treated with He Jie Tang (HJT) and 35 patients in group II were treated with combined medication. The levels of T-lymphocyte subsets (CD_3^+, CD_4^+, CD_8^+), NK cell activity, cytokines (TNF-α, IL-8, sIL-2R) were observed before and after the treatment. Another 20 normal persons served as group 3.

RESULTS: The level of CD_4^+ cells and NK cell activity were lower, whereas the level of CD_8^+ cells in patients was higher than that in normal persons (t = 2.685, 3.172, and 2.754 respectively; P<0.01). The levels of TNF-α, IL-8, and sIL-2R in chronic hepatitis B patients were higher than those in normal persons (t = 3.526, 3.170, and 2.876 respectively; P<0.01). After 6 months of treatment, ALT, AST, and TB levels in the two groups were obviously decreased (t = 3.421, 3.106, and 2.857 respectively; P<0.01). The level of CD_4^+ cells and NK cell activity were increased whereas the level of CD_8^+ cells decreased (t = 2.179, 2.423, and 2.677 respectively; P<0.05) in group I. The levels of TNF-α, IL-8, and sIL-2R in group I were decreased significantly after the treatment (t = 2.611, 2.275, and 2.480 respectively; P<0.05) but had no significant difference in group II after the treatment (t = 1.906, 1.833, and 2.029 respectively; P>0.05). The total effective rate had no significant difference between the two groups (χ^2 = 2.882, P>0.05) but the markedly effective rate was significantly different between the two groups (χ^2 = 5.340, P<0.05).

CONCLUSION: HJT is effective in treating chronic hepatitis B. HJT seems to exert its effect by improving the cellular immune function and decreasing inflammatory cytokines in chronic hepatitis B patients. The function of HJT in protecting liver function in the process of eliminating virus needs to be further studied.

Key words: He Jie Tang; Lymphocyte subsets; NK cell; Cytokines; Chronic hepatitis B

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INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a serious clinical problem worldwide and may lead to end-stage liver disease, cirrhosis, and hepatocellular carcinoma (HCC), *etc.*[1-3]. The pathogenesis of hepatitis B is very complex and has not been clarified. Generally, HBV itself does not directly damages hepatocytes, but results in dysfunction of cell-mediated immunity[3-5]. Peripheral blood mononuclear cells (PBMCs), which are aggregated immunologically competent cells, such as T lymphocytes, natural, and lymphokine-activated killer cells, likely play an important role in anti-HBV infection.

Some agents such as interferon (INF) and lamivudine have been proved to be effective for chronic hepatitis B, but their efficacy is limited to a small percentage of highly selected patients[6-13]. The management of chronic hepatitis B remains a clinical challenge.

Traditional Chinese medicine (TCM) has a long history in treating hepatitis, and has been proven to have good curative effects and fewer side effects in treating acute and chronic liver diseases. HJT is a recipe for chronic hepatitis B, which can improve liver function and immunity of chronic hepatitis B patients as the seroconversion rate of HBeAg[14]. In order to analyze the immunoregulatory mechanisms of HJT, we treated chronic hepatitis B patients with HJT from June 1999 to March 2003 and

observed the clinical effect of HJT on T lymphocyte subset level, NK cell activity as well as TNF-α, IL-8, and sIL-2R level.

MATERIALS AND METHODS

Patients

A total of 85 patients with chronic hepatitis B were enrolled in this study and randomly divided into two groups. There were 27 males and 23 females aged 18-60 years (mean 36.9±9.5 years) in group I. There were 19 males and 16 females aged 18-60 years (mean 38.5±9.1 years) in group II. The difference in clinical data between the two groups was insignificant. Twenty age-matched healthy donors from the Blood Center of our hospital were assigned as group III. This prospective study was approved by the local ethics committee and written consent was obtained from the participants.

Diagnostic criteria

Patients with a history of hepatitis B or HBsAg carriers for at least 6 mo, who still had symptoms and signs of hepatitis as well as abnormal liver function and positive HBsAg, HBeAg and HBV-DNA, were diagnosed as chronic hepatitis B in the present study.

Criteria for enrollment

Patients, aged 18-60 years with their serum alanine aminotransferase (ALT) level being 80-240 μ/L and who had positive serum HBeAg and HBV-DNA, were enrolled. The diagnosis of hepatitis B was made in accordance with the standards for chronic viral hepatitis issued in the Fifth National Conference on Infectious Diseases and Parasitosis (Beijing, China, 1995).

Criteria for exclusion

Patients aged over 60 years or less than 18 years, patients in pregnancy or in breast feeding period; patients who had hepatitis C or other hepatic viral infection, autoimmune hepatitis and drug-induced hepatitis or alcoholic hepatitis; patients with severe complications of the cardiovascular, renal or hematopoietic system and patients with mental diseases, were excluded.

Group I was treated with HJT that consisted of 10 g Radix Bupleuri, 12 g Radix Scutellariae, 9 g Rhizoma Pinelliae, 30 g Radix Codonopsis Pilosulae, 6 g Radix Glycyrrhizae Praeparata, 9 g Fructus Ziziphi Jujubae, 30 g Rhizoma Polygoni Cuspidati, 8 g Radix Morindae Officinalis, 30 g Herba Hedyotis Diffusae. One dose was taken per day for 6 mo. Group II was treated with oxymatrine (200 mg, t.i.d.), compound vitamin B (2 tablets, t.i.d.), vitamin C (100 mg, t.i.d.), vitamin E (50 mg, t.i.d.), and ester capsule (2 tablets, t.i.d.) for 6 mo.

Patients who had normal serum ALT and sero-conversion of HBeAg and HBV DNA (quantitative PCR) after treatment were defined as responders while those with negative results as non-responders.

Recording and observation of symptoms and signs

The symptoms and signs of patients were recorded in detail using the "Clinical Observation Table" once a month before and during the treatment.

Etiological markers of hepatitis B

HBV-M and anti-HAV, anti-HCV, anti-HDV, and anti-EBV marks were detected by enzyme-linked immunosorbent assay (ELISA). HBV-DNA was detected by quantitative polymerized chain reaction (PCR).

Liver function

The patients had liver function examination every month during the treatment, including contents of serum proteins, total bilirubin (TB) and activities of ALT and AST (aspartate aminotransferase).

T-lymphocyte subsets and NK cell activity

T-lymphocyte subsets were detected by the single clone antibody APAAP method, NK cell activity was assayed by MTT colorimetry.

Detection of cytokines

The levels of TNF-α, sIL-2R, and IL-8 were detected by double antibody sandwich ELISA.

Statistical analysis

All statistical analyses were performed by χ^2 test and Wilcoxon rank sum test using SPSS software. $P<0.05$ was considered statistically significant.

RESULTS

Standard for efficacy evaluation

The clinical efficacy of treatment was evaluated according to the following standards. Markedly effective: chief symptoms including right upper abdomen pain, poor appetite, and abdominal distention disappeared; HBeAg and HBV-DNA turned negative; serum levels of ALT, AST, and TBIL restored to normal. Effective: chief symptoms were alleviated or improved; the level of HBV-DNA decreased; HBeAg did not turn negative; serum levels of ALT, AST, and TBIL decreased by >50% of the original levels. Ineffective: the chief symptoms or the serum levels of ALT, AST, and TBIL or HBeAg and HBV-DNA did not show any improvement.

Clinical efficacy of treatment

In group I, treatment was markedly effective in 7 cases, effective in 41 and ineffective in 2, the total effective rate being 96.0%. In group II, treatment was markedly effective in 0 cases, effective in 30, and ineffective in 5, the total effective rate being 85.7%. The difference in total effective rate was insignificant between the two groups ($P>0.05$) and the markedly effective rate was significantly different between the two groups ($P<0.05$).

Levels of ALT, AST, TB, and HBV-DNA before and after the treatment

After 6 mo of treatment, the levels of ALT, AST, and TB in two groups were obviously decreased ($P<0.01$). HBV-DNA level in group I was obviously decreased ($P<0.05$). HBV-DNA and HBeAg turned negative in seven patients and HBeAg turned negative in two patients but HBV-DNA did not turn negative. HBeAg turned negative in two patients of group II but HBV-DNA did not turn negative (Table 1).

T lymphocyte subsets before and after the treatment

The level of CD_4^+ cells was lower whereas the level of CD_8^+ cells (groups I and II) was higher in patients than in normal persons (group III) ($P<0.01$). There was no significant difference between the levels of CD_3^+ cells in patients and normal persons ($P>0.05$). After 6 mo of treatment, the level of CD_4^+ cells increased, whereas the level of CD_8^+ cells decreased ($P<0.05$) in group I. However, the levels of CD_4^+ and CD_8^+ cells had no significant difference in group II ($P>0.05$, Table 2).

Serum levels of TNF-α, sIL-2R, and IL-8 as well as NK activity before and after the treatment

The NK cell activity was lower whereas the levels of TNF-α, sIL-2R, and IL-8 was higher in patients (groups I and II) than in normal persons (group III) ($P<0.01$). After 6 mo of treatment, NK cell activity was significantly increased, whereas the levels of TNF-α, sIL-2R, and IL-8 decreased ($P<0.05$) in group I. However, there was no significant difference in group II ($P>0.05$, Table 3).

T lymphocyte subsets and NK activity of responders and non-responders of group I before and after the treatment

The levels of CD_3^+, CD_4^+, and CD_8^+ cells and NK cell activity in the two groups had no significant difference before treatment ($P>0.05$). After 6 mo of treatment, the level of CD_4^+ cells and NK cell activity increased, whereas the level of CD_8^+ cells decreased in responders ($P<0.05$). NK cell activity and the level of CD_4^+ and CD_8^+ cells in the non-responders had no significant difference after treatment ($P>0.05$, Table 4).

DISCUSSION

Though the pathogenesis of chronic hepatitis B remains unclear, a great many studies have shown that chronic hepatitis B patients are usually accompanied with disorder of immune function and hepatocyte damage is mainly caused by immunological injury[15–19]. Alterations of T

Table 1 Levels of ALT, AST, TB, and HBV-DNA before and after the treatment (mean±SD)

		n	ALT (U/L)	AST (U/L)	TB (μmol/L)	HBV-DNA (copy/mL)
Group III		20	21.52±8.90	15.56±7.65	11.75±5.71	<1 000
Group I	Pre-T	50	232.52±12.25	139.65±9.62	43.35±5.86	$(1.62\pm0.81)\times10^{8.31}$
	Post-T	50	33.26±9.35[b]	35.18±8.26[b]	19.95±5.12[b]	$(9.25\pm1.90)\times10^{5.02a}$
Group II	Pre-T	35	225.70±11.61	135.45±9.21	41.45±5.85	$(1.47\pm0.65)\times10^{8.22}$
	Post-T	35	30.86±8.95[b]	65.68±8.82[b]	29.55±546[b]	$(8.26\pm2.20)\times10^{7.62}$

Pre-T: before treatment; Post-T: after treatment; [a]$P<0.05$ *vs* before treatment in the same group; [b]$P<0.01$ *vs* before treatment in the same group.

Table 2 T lymphocyte subsets before and after the treatment (mean±SD)

		n	CD_3(%)	CD_4(%)	CD_8(%)	CD_4/CD_8
Group III		20	68.10±9.25	39.27±8.70	30.96±6.82	1.70±0.72
Group I	Pre-T	50	65.55±8.22	35.06±5.38[b]	34.80±4.36[b]	1.10±0.35[b]
	Post-T	50	67.35±8.85	37.60±8.52[a]	31.95±5.61[a]	1.31±0.42[a]
Group II	Pre-T	35	65.86±9.21	35.15±6.01[b]	35.10±6.56[b]	1.07±0.46[b]
	Post-T	35	66.71±9.56	35.92±8.55	34.66±6.25	1.12±0.36

Pre-T: before treatment; Post-T: after treatment; [b]$P<0.01$ *vs* group III ; [a]$P<0.05$ *vs* before treatment in the same group.

Table 3 Serum levels of TNF-α, sIL-2R, and IL-8 as well as NK activity before and after the treatment (mean±SD)

		n	TNF-a (mg/L)	sIL-2R (kU/L)	IL-8 (μg/L)	NK (%)
Group III		20	0.58±0.23	310.0±30.7	0.72±0.2	59.65±7.5
Group I	Pre-T	50	18.8±8.9[b]	390.9±12.0[b]	2.42±0.8[b]	43.12±6.5[b]
	Post-T	50	10.5±6.8[a]	310.22±8.9[a]	1.12±0.5[a]	52.90±7.0[a]
Group II	Pre-T	35	19.0±7.2[b]	395.7±16.5[b]	2.45±0.8[b]	43.02±6.8[b]
	Post-T	35	15.62±7.9	355.6±9.5	1.80±0.7	46.54±6.9

αPre-T: before treatment; Post-T: after treatment; [a]$P<0.05$ *vs* before treatment in the same group; [b]$P<0.01$ *vs* group III.

Table 4 T lymphocyte subsets in responders and non-responders of group I before and after the treatment (mean±SD)

		n	CD_3 (%)	CD_4 (%)	CD_8 (%)	NK (%)
Responders	Pre-T	7	66.02±8.86	35.10±4.76[b]	34.92±4.36[b]	43.52±7.1[b]
	Post-T	7	67.80±9.11	38.85±8.85[a]	30.15±5.82[a]	55.60±8.2[a]
Non-responders	Pre-T	43	65.50±9.08	34.92±6.30[b]	34.77±6.56[b]	42.93±6.7[b]
	Post-T	43	66.09±9.35	35.99±8.70	34.25±5.52	45.60±6.5
Group III		20	68.10±9.25	39.27±8.70	30.96±6.82	59.65±7.5

[a]$P<0.05$ *vs* before treatment in the same group; [b]$P<0.01$ *vs* group III.

lymphocyte subsets and NK cells are important reasons for the disorder of immune function due to HBV infection, TNF-α, IL-8, and sIL-2R are important cytokines associated with liver damage. Therefore, the importance of T lymphocytes and NK cells as well as cytokines in the occurrence of chronic HBV infection has received more and more attention.

CD_3^+, CD_4^+, and CD_8^+ cells are major function subgroups of T cells. An antiviral cellular immune response of CD_4^+ and CD_8^+ is the important mechanism of hepatocyte injury induced by HBV, the specific response of CD_4^+ and CD_8^+ to the virus antigen is closely related with the elimination of the virus[6,20,21]. NK cells play a critical role in host innate defense against viruses and are partly responsible for liver injury in the process of erasing viruses[22-28]. Recent studies found that NK cells are potent activators of dendritic cells (DCs), which have an impact on the magnitude and direction of DC activation of T cells under the conditions of chronic viral infection, activated NK cells can release cytokines and prevent virus from reproducing[23,29]. Therefore, T-lymphocyte subsets and NK activity can be considered as an appropriate response of immune system to inhibit viral replication and HBV eradication. In the present study, we discovered that in the outbreak period of chronic hepatitis B, NK activity and level of CD_4^+ cells were lower, whereas the level of CD_8^+ cells was higher in patients than in normal persons, suggesting that disorders of cellular immune function and pathologic damages occur in chronic hepatitis B patients.

The serum NK activity and CD_4^+ cell level in non-responders were lower than those in normal persons, whereas the level of CD_8^+ cells in non-responders was higher than that of normal persons. After treatment, the NK activity and CD_4^+ cell level were increased in seven patients with the conversion of HBV-DNA and HBeAg and the liver function resumed to normal. The results suggest that T-lymphocyte subsets and NK activity are depressed rather than activated in viral hepatitis B, but levels of T lymphocyte subsets and NK activity are closely related with different courses of hepatitis B. At the same time, levels of T lymphocyte subsets and NK activity in some patients were still low in palliative period, indicating that the chance of recrudescence might increase. T lymphocyte subsets and NK cells play a critical role in response to HBV infection and their level and mutual relation can be used to identify the cellular immune level in patients with chronic hepatitis B[11,38].

TNF-α plays an indispensable role in liver injury mediated by specific immune response to HBV infection[30]. Pretreatment with anti-TNF-α mAb in animal model strongly blocks Th1 cell-induced hepatocyte necrosis and apoptosis[32]. However, it was reported that TNF-α exerts its antiviral effects without destruction of hepatocytes[33]. IL-8 is a chemotactic factor of neutrophils and T cells and plays a role in hepatic injury in patients with chronic viral hepatitis. Remarkable increase of IL-8 leads to accumulation of cytotoxic T lymphocytes, which get direct and immediate access to the target hepatocytes and the resident intrahepatic macrophages, subsequently causing the damage of hepatocytes[34-36]. Release of sIL-2R from activated T lymphocytes may occur as a result of proteolysis of mIL-2R or as a result of alternative mRNA process. High level of sIL-2R in chronic HBV infection appears directly related to the activity of liver diseases; therefore, serum sIL-2R levels can be used to indicate the degree of liver damage in patients with chronic HBV infection[31,37,38].

In the present study, we discovered that in the outbreak period of chronic hepatitis B, the levels of IL-8, TNF-α, and sIL-2R were higher in patients than in normal persons during and after HJT treatment, significantly increased suggesting that cytokines and immunocytes may play a role in the pathogenesis of chronic hepatitis B.

HJT is a recipe for treating hepatitis in which cold and warm drugs are used to eliminate evils and restore healthy energy. Former research indicates that HJT can protect the liver from injury[20,21]. We discovered that HJT could improve liver function and NK activity, regulate T cellular immune function in chronic hepatitis B patients. The results suggest that HJT exerts its effect by improving the cellular immune function and decreasing inflammatory cytokines in chronic hepatitis B patients.

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