

Effect of Hejie decoction on T cell immune state of chronic hepatitis B patients

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Abstract

AIM: To explore the effect of Hejie decoction (HJD) (mediation decoction) on T cellular immune state of chronic hepatitis B patients.

METHODS: Sixty-five patients with chronic hepatitis B were randomly divided into 2 groups. Forty patients in the treatment group were treated by HJD, and 25 patients in the control group were treated by routine Western medicine. The TCRV β_7 gene expression, T lymphocyte subsets (CD $_3^+$, CD $_4^+$, CD $_8^+$, CD $_4$ /CD $_8^+$) levels were observed before and after treatment.

RESULTS: The level of CD $_4^+$ cells was lower whereas the level of CD $_8^+$ cells was higher in patients than in the normal group. There was no significant difference between the levels of CD $_3^+$ cells in patients and normal persons. After 6 months of treatment, ALT, AST, TB levels of the 2 groups were obviously decreased, and the level of CD $_4^+$ cells was increased whereas the level of CD $_8^+$ cells was decreased in the treatment group. However, the level of CD $_4^+$ cells and CD $_8^+$ cells had no significant difference in the control group. TCRV β_7 expressions were detected in 6 patients of the treatment group, whose HBV-DNA and HBeAg turned negative and ALT became normal. HBeAg in another 3 patients turned negative while HBV-DNA did not, and TCRV β_7 expressions were not detectable. TCRV β_7 expression could not be detected in the control group, HBV-DNA of the control group did not turn negative. HBeAg in 1 patient turned negative while HBV-DNA did not, and TCRV β_7 expressions were not detectable. The total effective rate was not significantly different between the 2 groups and the markedly effective rate was significantly different ($P < 0.01$).

CONCLUSION: HJD is effective for treating chronic hepatitis B, and its effect seems to relate with the improvement of the TCRV β_7 expression of chronic hepatitis B patients, thus activating T cells and eliminating HBV. T cellular immune function plays an important role in HBV infection and virus elimination.

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INTRODUCTION

T cells take charge of recognizing the cells infected with bacilli and virus, as well as cancer cells^[1]. Recent studies have demonstrated that the stimulating signal would be transferred to the inside of cells by CD $_3$ molecules when antigens are recognized by T cell receptor (TCR), sequentially activating T lymphocyte cells. TCR plays a crucial role in exerting T cellular immune function. Therefore it is very important to investigate the relation of T cellular function and clinical effect by studying the function of T cell receptor^[2-4]. We treated chronic hepatitis B patients with HJD from June 1999 to March 2003, and observed the relation between clinical effects and T lymphocyte subsets, TCRV β_7 gene expression. The results are reported as follows.

MATERIALS AND METHODS

Materials

All the 65 patients with chronic hepatitis B enrolled were outpatients from the special clinics of liver diseases, and were divided into 2 groups according to random number table. The 40 patients in the treatment group were 22 males and 18 females, aged 18-60 years, averaged 38.6 \pm 9.8 years, with an average course of illness of 0.8-12.5, 3.5 \pm 1.2 years. The 25 patients in the control group were 14 males and 11 females, aged 18-60 years, averaged 39.0 \pm 8.9 years, with an average course of illness of 0.6-11.1, 3.2 \pm 1.1 years. The difference of clinical data between the 2 groups was insignificant, so the 2 groups were comparable. Ten age-matched healthy donors from the Blood Center of our hospital were assigned as normal group.

Diagnostic criteria

The patient had a history of hepatitis B or HBsAg carrier for at least 6 mo and still had the symptoms and signs of hepatitis and abnormal liver function at the time when they were included in the trial. Their HBsAg, HBeAg and HBVDNA were positive.

Criteria of enrollment

The patients were aged 18-60 years, their serum levels of alanine aminotransferase (ALT) were between 80 U/L and 240 U/L, their serum HBeAg and HBV-DNA (quantitative PCR) were positive. The diagnostic criteria of hepatitis B were in accordance with the standards for chronic viral hepatitis issued in the Fifth National Conference on Infectious Diseases and Parasitosis in China (Beijing, China, 1995)^[5].

Criteria of exclusion

The patients who were over 60, or less than 18 years old, and those who were pregnant or in breast-feeding period, those who were complicated with hepatitis C or other hepatic viral infection, suspicious of autoimmune hepatitis, and drug hepatitis or alcoholic hepatitis, as well as those who had severe

complications of cardiovascular, renal or hematopoietic systems and mental diseases were excluded from the trial.

Methods

The treatment group was treated with Hejie decoction, consisting of Radix Bupleuri 10 g, Radix Scutellariae 12 g, Rhizoma Pinelliae 9 g, Radix Codonopsis Pilosulae 30 g, Radix Glycyrrhizae Praeparata 6 g, Fructus Ziziphi Jujubae 9 g, Rhizoma Polygoni Cuspidati 30 g, Radix Morindae Officinalis 8 g, Herba Hedyotis Diffusae 30 g. One dose was taken per day for 6 mo. The control group was treated with compound vitamin B₂, 2 tablets, vitamin C 100 mg, vitamin E 50 mg, Wuzhi capsules, 2 tablets, 3 times a day for 6 mo. Patients who had normal serum ALT, HBeAg and HBV DNA (quantitative PCR) after treatment were defined as responders, while those with negative results were taken as non-responders.

Patients' symptoms and signs were recorded in detail using "Clinical Observation Table" once a month before and during the treatment. HBV-M and anti-HAV, anti-HCV, anti-HDV and anti-EBV marks: Enzyme linked immunosorbent assay (ELISA) kit was obtained from Shanghai Kehua Corporation. HBV-DNA: Quantitative polymerized chain reaction (PCR) kit was from Diagnostic Center of Sun Yat-Sen University.

The patients had liver function examination (American Experiment Instrument Corporation) every month during the treatment, including contents of serum proteins, total bilirubin (TB), and activities of ALT and AST (aspartate aminotransferase). The kit was a product of American Experiment Instrument Corporation.

T-lymphocyte subsets were detected using single clone antibody APAAP method, the kits were purchased from Wuhan Boster Biological Technology Co. Ltd.

Peripheral blood mononuclear cells (PBMC) were prepared from 8 mL of freshly, heparinized blood by centrifugation at 400 r/min through a Ficoll-hypaque density gradient, washed 3 times with 10 g/L BSA in PBS and resuspended in 5 g/L BSA in PBS, stored on ice or at -70 °C for extraction of RNA.

Total cellular RNA was extracted from fresh PBMC with acid guanidinium thiocyanate-phenol-chloroform extraction according to the manufacturer's instructions, RNAs were purchased from Promega Corporation.

The primers were synthesized on an applied biosystems DNA synthesizer (Shanghai Shengong Company, China), and the sequences^[6] were (5' -3'): CCTGAATGCCCAACAGCTCTC,

expanding length: 235. β -actin was prepared as an internal standard to quantify the products. Three micrograms of total RNA was used to synthesize first-strand cDNA. RT-PCR was carried out according to the manufacturer's instructions (Promega, USA). The amplified products were then electrophoresed on 20 g/L agarose gel. The electrophoresis images were scanned by Fluor-S MultiImager (Bio-Rad, USA) and analyzed according to the V β / β -actin ratios by computing densitometer and Image Quant software.

Statistical analysis

All statistics were performed by using statistical procedure of social science (SPSS), including chi-square test and Wilcoxon rank sum test. The probability values less than 0.05 were considered significant.

RESULTS

Standard for efficacy evaluation

The clinical efficacy of treatment was evaluated according to the following standards formulated by authors: (1) 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, TBIL restored to normal. (2) Effective: Chief symptoms were alleviated or improved, the level of HBV-DNA decreased, HBeAg did not turn negative, serum levels of ALT, AST, TBIL decreased by >1/2 of the original levels. (3) Ineffective: Neither the chief symptoms nor the serum levels of ALT, AST and TBIL or HBeAg, HBV-DNA showed any improvement.

Clinical efficacy of treatment

In the treated group, the treatment was markedly effective in 6 cases, effective in 33 and ineffective in 1, the total effective rate was 97.5%. In the control group, the treatment was markedly effective in 0 cases, effective in 22 and ineffective in 3, the total effective rate was 88.0%. The difference of total effective rate was insignificant between the 2 groups ($P>0.05$) and that of markedly effective rate was significant ($P<0.01$).

ALT, AST, TB and HBVDNA levels before and after treatment, as well as TCRV β gene expression

After 6 mo of treatment, the ALT, AST, TB levels of the 2 groups

Table 1 ALT, AST, TB and HBVDNA levels before and after treatment (mean \pm SD)

	<i>n</i>	ALT (U/L)	AST (U/L)	TB (μ mol/L)	HBVDNA (copy/mL)
Normal	10	27.80 \pm 8.65	19.07 \pm 8.50	12.55 \pm 5.52	0
Treatment					
Pre-T	40	213.66 \pm 10.30	134.66 \pm 9.82	41.03 \pm 4.36	(1.52 \pm 0.72) $\times 10^{8.25}$
Post-T	40	37.01 \pm 9.75 ^b	29.07 \pm 8.97 ^b	20.55 \pm 5.52 ^b	(4.25 \pm 1.90) $\times 10^{6.02}$ ^a
Control					
Pre-T	25	195.70 \pm 11.11	125.12 \pm 9.21	40.87 \pm 6.78	(1.32 \pm 0.89) $\times 10^{8.12}$
Post-T	25	36.01 \pm 9.75 ^b	69.88 \pm 8.97 ^b	30.55 \pm 5.52 ^b	(6.95 \pm 2.39) $\times 10^{7.82}$

n: number; normal: normal group; treatment: treatment group; control: control group; 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 treatment (mean \pm SD)

	<i>n</i>	CD ₃ (%)	CD ₄ (%)	CD ₈ (%)	CD ₄ /CD ₈
Normal	10	67.80 \pm 8.65	39.07 \pm 8.50	30.55 \pm 5.52	1.62 \pm 0.46
Treatment					
Pre-T	40	65.97 \pm 8.45	34.76 \pm 4.82 ^b	34.08 \pm 4.36 ^b	1.04 \pm 0.32
Post-T	40	67.01 \pm 9.75	37.39 \pm 8.97 ^a	32.35 \pm 5.52 ^a	1.20 \pm 0.30 ^a
Control					
Pre-T	25	65.70 \pm 9.11	35.02 \pm 5.21 ^b	34.87 \pm 6.78 ^b	1.02 \pm 0.29
Post-T	25	66.01 \pm 9.75	35.88 \pm 8.97	34.15 \pm 5.52	1.09 \pm 0.39

n: number; normal: normal group; treatment: treatment group; control: control group; Pre-T: before treatment; Post-T: after treatment; ^a $P<0.05$, vs before treatment in the same group, ^b $P<0.01$, vs control.

were obviously decreased ($P<0.01$), HBVDNA levels of the treatment group were obviously decreased ($P<0.05$, Table 1). TCRV β_7 expressions were detected in 6 patients of the treated group, and their HBV-DNA and HBeAg turned negative, and HBeAg in another 3 patients turned negative, but HBV-DNA did not turn negative, and TCRV β_7 expressions were not detectable. The TCRV β_7 expression could not be detected in the control group, HBV-DNA of the control group did not turn negative. HBeAg in 1 patient turned negative in the control group, but HBV-DNA did not turn negative, and TCRV β_7 expressions were not detectable. In patients without HBeAg negative conversion, or patients with HBeAg negative conversion and positive HBV-DNA and normal liver function, TCRV β_7 expression could not be detected.

T lymphocyte subsets before and after treatment

The level of CD $_4^+$ cells was lower whereas the level of CD $_8^+$ cells was higher in the patients than in the normal group ($P<0.01$), there was no significant difference between the levels of CD $_3^+$ cells of the patients and normal persons. After 6 mo of treatment, the level of CD $_4^+$ cells increased whereas the level of CD $_8^+$ cells decreased ($P<0.05$) in the treated group. However, the level of CD $_4^+$ cells and CD $_8^+$ cells had no significant difference in the control group ($P>0.05$, Table 2).

Table 3 T lymphocyte subsets of responders and non-responders in treatment group before and after treatment (mean \pm SD)

		<i>n</i>	CD $_3$ (%)	CD $_4$ (%)	CD $_8$ (%)
Responders	Pre-T	6	66.62 \pm 8.86	35.10 \pm 4.76 ^b	34.02 \pm 4.86 ^b
	Post-T	6	66.80 \pm 9.11	38.85 \pm 8.85 ^a	30.15 \pm 5.82 ^a
Non-responders	Pre-T	34	65.86 \pm 9.08	34.62 \pm 6.30 ^b	34.17 \pm 6.56 ^b
	Post-T	34	66.09 \pm 9.35	35.72 \pm 8.70	33.85 \pm 5.52
Normal		10	67.80 \pm 8.65	39.07 \pm 8.50	30.55 \pm 5.52

n: number; responders: patients who had normal ALT and HBeAg and HBV DNA after treatment; non-responders: patients who had abnormal ALT, HBeAg and HBV DNA after treatment; normal: normal group; Pre-T: before treatment; Post-T: after treatment; ^a $P<0.05$, vs before treatment in the same group, ^b $P<0.01$, vs normal group.

T lymphocyte subsets of responders and non-responders in treatment group before and after treatment

The level of CD $_3^+$, CD $_4^+$, CD $_8^+$ cells in the 2 groups had no significant difference before treatment ($P>0.05$). The level of CD $_4^+$ cells increased whereas the level of CD $_8^+$ cells decreased in the responders' group ($P<0.01$). The level of CD $_4^+$ and CD $_8^+$ cells in the non-responders' group had no significant difference after treatment ($P>0.05$, Table 3).

DISCUSSION

Although the pathogenesis of chronic hepatitis B has not been fully studied, the importance of cellular immune in the occurrence of chronic HBV infection and elimination of HBV has received more and more attention^[7]. CD $_3^+$, CD $_4^+$ and CD $_8^+$ are the major function subsets of T cells, many studies have discovered that CD $_3^+$, CD $_4^+$ of chronic hepatitis B with serum HBV positive are lower than those of chronic hepatitis B with serum HBV negative, and the higher the quantity of HBVDNA is, the lower the T cellular immune function is. 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. It is suggested that T cells could play a critical role in response to HBV infection, and their level and mutual relationship could be used to identify the cellular immune

level and could serve as one of the valuable immunologic targets to forecast the change of patients' condition^[8-12]. Some studies on chronic hepatitis B showed that T cell receptor function was the important cause of the obstacle to T cells, sequentially bringing about HBV escaping immune response, and finally resulting in standing HBV infection^[2,3,13-16]. Therefore, it is very important to study the antiviral effect of T cells starting with immune identification.

TCR has been found to be the receptor of T lymphocyte surface recognising extra antigen and the major histocompatibility complex I (MHC I), as well as the specific sign of T cells^[17-19]. The genes of the α and β chains which promote TCR α β reset formation of large amount of specific TCR to recognise the extra antigen when they meet antigen. TCR and CD $_3$ would inosculate on the surface cells and form TCRCD3 compounds, the stimulating signals would be transferred to the inside of cells by CD3 molecules when antigens were recognised by TCR, activating T lymphocyte cells^[20-26]. Some studies have indicated that the priority expression and employment of TCRV β_7 were related with the specific immune reaction of chronic hepatitis B^[27-29]. Therefore, It is a meaningful pathway to eliminate HBV and decrease the occurrence of liver cancer by screening experimental recipes to activate T cells by improving T cell recognising function under the present circumstances of low cure ratio and high recurrence. We discovered that in the outbreak period of chronic hepatitis B, TCRV β_7 gene expression was low. The level of CD $_4^+$ cells was lower whereas the level of CD $_8^+$ cells was higher in patients than in the normal group ($P<0.01$). The serum TCRV β_7 gene expressions of non-responders were low, the level of CD $_4^+$ cells of non-responders was lower in patients than in normal persons whereas the level of CD $_8^+$ cells of non-responders was higher in patients than in the normal persons. After treatment, the TCRV β_7 gene expression was high in patients with the conversion of HBV-DNA and HBeAg, and the liver function, the level of CD $_4^+$ and CD $_8^+$ cells resumed to normal. The levels of CD $_4^+$ and CD $_8^+$ cells in the treatment group were significantly different from those before treatment ($P<0.01$). The results showed that TCRV β_7 participated in the elimination of HBV and cytotoxic function, and the occurrence of chronic hepatitis B was related to the low expression of TCRV β_7 . The CTL could not be effectively activated to kill or injure HBV due to the obstacle of T cell receptor function, sequentially bringing about stable reproduction of HBV and resulting in chronic inflammation of hepatocytes. At the same time, we also discovered that TCRV β_7 expression of some patients in the palliating period was still low, indicating that recurrence might increase.

When HBeAg in chronic hepatitis B patients transforms to Anti-HBe, hepatocyte injury aggravates, which is considered to be mediated by CTL antiviral cellular immune response. The dynamic observation of 6 cases whose TCRV β_7 gene expressions were positive showed that the TCRV β_7 gene expression related with the HBeAg serum conversion, the decreased quantity of HBVDNA, and ALT levels experienced a period of acute exacerbation in the course of descending, suggesting that TCRV β_7 participated in the elimination of HBV and cytotoxic function. ALT fluctuation in patients with TCRV β_7 negative was small, and there was no obvious decrease in HBVDNA, or obvious HBeAg serum conversion, suggesting the cellular immune response of the patients was feeble. Thus TCRV β_7 might be an index to evaluate the cellular immune state of hepatitis B patients.

HBeAg is a good index that reflects the HBV replication. When HBeAg transforms to Anti-HBe, the reproduction of HBV will obviously weaken or cease, along with the relief of the state of illness. Among the 10 patients with HBeAg serum conversion, the quantity of HBVDNA in some patients did not alleviate or vanish after HBeAg serum conversion, and the

state of illness fluctuated, which should be treated continually. HJD is a proved recipe for treating hepatitis, in which cold and warm drugs are used to eliminate evils and restore healthy energy. Previous researches indicated that HJD had the effect to protect the liver, as well as the function of antiviral and immune regulation^[30-33]. We discovered that HJD could meliorate liver function, regulate TCRV β 7 gene expression, improve T cellular immune function of chronic hepatitis B in this study. The results suggest that the clinical effects of HJD on chronic hepatitis B, especially on the elimination of HBV, might relate with the improvement of TCR identifying function, and effectively activate CTL. However, HJD could not interrupt immune endurance of some patients, resulting in the failure of treatment, which needs further study to explore the cause and resolving methods.

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