Effect of IFN-A on immune responses and plasmacytoid dendritic cell functions of patients with hepatitis B.

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Abstract

Objective: To explore the impact of IFN-α on immune response of patients with hepatitis B and the functions of plasmacytoid Dendritic Cells (pDCs).

Methods: A total of 96 patients with hepatitis B were involved in the study and randomly assigned into control group and experimental group, 48 a group. Both groups underwent usual treatment, besides, the experimental group was given IFN-α. After treatment, all patients were tested for serum AST, ALT and IFN-α, as well as peripheral CD4+, CD8+, CD4+/CD8+, and pDCs.

Results: Compared with the control group, the peripheral CD4+ and CD4+/CD8+ of the experimental group after treatment were much more than those of the control group. Serum IFN-αand peripheral pDCs of the experimental group were also more than that of the control group (P<0.05); while serum AST and ALT of the experimental group were much less than that of the control group (P<0.05).

Conclusion: IFN-α is able to greatly enhance the immune responses of patients with hepatitis B and functions of plasmacytoid dendritic cells, improving liver function.

Introduction

Hepatitis B Virus (HBV) infection is a global healthy issue, and approximate 2 billion people worldwide have been infected with HBV, of which 350-400 million are chronic HBV infection, and about 1 million persons die of HBV infection-induced liver failure, liver cirrhosis and primary hepatocellular carcinoma every year [1]. Hepatitis B is an infectious disease caused by HBV, with liver inflammatory lesions, which can give rise to multi-organs damage [2]. As a high incidence area of hepatitis B, China has more than 20 million patients with Chronic Hepatitis B (CHB) at present, and hepatitis B-related diseases result in 237, 000 deaths every year, of which 156, 000 die of liver cancer [3], with an uptrend in incidence year by year. CHB has strong infectivity and gets a relapse easily which develops into liver cirrhosis or even liver cancer, badly threatening patients' lives. Therefore, a proper and effective treatment is essential for CHB patients at early stage. It is internationally recognized that IFN-α and nucleoside analogues are the main drugs for the anti-virus therapy of chronic hepatitis B. IFN-α is the sole drug approved for the anti-virus therapy of hepatitis B and immunoregulation that can stimulates the body to produce immune responses with HBV-specificity. Compared with nucleoside analogues, IFN-α cannot lead to drug-fast virus and has a relatively definite

treatment course. Consequently, the study observes the changes of Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), and IFN-α in serum and CD8+ T helper cells (CD8+), CD4+ T helper cells (CD4+), CD4+/CD8+, and plasmacytoid Dendritic Cells (pDCs) in peripheral blood of patients before and after treatment to investigate the effect of IFN-α on the immune response of patients with hepatitis B and functions of pDCs.

Data and Methods

Clinical data

A total of 96 patients with CHB diagnosed in infection department from December 2015 to December 2016 were enrolled in the study and divided randomly into control group and experimental group, 48 a group. In the experimental group, there were 25 males, 23 females, aged from 22 to 70 y, (41.6 ± 11.5 y) in average, with a mean course of (9.1 ± 3.8 months). And in the control group, there were 24 males, 24 females, aged from 24 to 68 y, (39.8 ± 12.3 y) in average, with a mean course of (9.5 ± 4.2 months). Compared two groups, there was no statistical difference in general clinical data like sex, age, and mean course of disease. The study had been approved by ethnic committee and all participants signed an informed

consent. All patients were diagnosed in the light of the diagnosis standards of CHB in Guidelines for Preventing and Treating of Chronic Hepatitis B (2010) drafted by Chinese Society of Hepatology in 2010 [4] and experimental outcomes. If patients had negative HbeAg and other chronic liver diseases, hepatitis C and D, autoimmune liver disease, heart failure, renal dysfunction, and malignant tumors, and if patients took anti-viral drugs recently, and if patients were allergic to the drugs used in the study, they all were ineligible to the study.

Methods

Treatment methods: According to the guidelines for clinical medication, all patients were given usual liver protectants and symptomatic treatments for one year. In addition, the patients in the experimental group were injected IFN-α intramuscularly, $500\text{-}600 \times 10^4$ IU each time, with a treatment course of 48 w, once a week until the end.

Collection of serum indexes: All patients on an empty stomach for 12 h at least were collected 5 ml venous blood before and after treatment that were centrifuged for serums of which ALT and AST were tested by performance rate method. And IFN-α in serum was determined with a full functional ELISA provided by BioTek Synergy, American, of which operations were strictly in the light of the introductions of IFN-α assay kit.

Cytology assay: 3 ml fasting venous blood drawn from each patient before and after treatment were put into an anticoagulation tube and detected for peripheral CD4+, CD8+, CD4+/CD8+, and pDCs with Gallios flow cytometry from Beckman Coulter, Inc.

Statistical analysis

All data were analysed by software SPSS20.0. The outcomes were expressed by mean ± SD, and t-test was used for the comparison between groups, while one-way ANOVA for the comparison within groups. When P was less than 0.05, the difference was statistically significant.

Results

Comparison on peripheral CD4+, CD8+ and CD4+/CD8+ of two groups before and after treatment

After treatment, peripheral CD4+, CD8+ and CD4+/CD8+ of two groups were greatly increased, and the increases of CD4+ and CD4+/CD8+ in the experimental group was much more than those in the control group (P<0.05); while compare two groups, there was no statistical difference in CD8+ after treatment (P<0.05, Table 1).

Table 1. *Comparison on peripheral CD4+, CD8+ and CD4+/CD8+ of two groups before and after treatment.*

Group	n	Time	CD4+ (%)	CD8+ (%)	CD4+/CD8+ (%)
Control group	48	Before treatment	31.81 ± 3.84	28.37 ± 3.31	1.12 ± 0.17
		After treatment	34.13 ± 4.21*	27.67 ± 3.51	1.25 ± 0.15*
Experimental group	48	Before treatment	31.43 ± 4.11	28.27±3.51	1.11 ± 0.13
		After treatment	45.13 ± 5.13*#	27.91 ± 3.79	1.61 ± 0.16*#

*P<0.05, compared with the same group before treatment, #P<0.05, compared with the control group after treatment.

Comparison on serum IFN-α and peripheral pDCs of two groups before and after treatment

After treatment, the serum IFN-α and peripheral pDCs of two groups went up largely; and compared two groups, the serum IFN-α and peripheral pDCs of the experimental group raised more than that of the control group (P<0.05, Table 2).

Table 2. *Comparison on serum IFN-α and peripheral pDCs of two groups before and after treatment.*

Group	n	Time	IFN-α (ng/L)	pDCs (%)
Control group	48	Before treatment	3.15 ± 0.51	0.25 ± 0.02
		After treatment	3.81 ± 0.32*	0.28 ± 0.03*
Experimental group	48	Before treatment	3.23 ± 0.41	0.24 ± 0.03
		After treatment	6.21 ± 0.56*#	0.45 ± 0.04*#

*P<0.05, compared with the same group before treatment, #P<0.05, compared with the control group after treatment.

Comparison on serum AST and ALT of two groups before and after treatment

After treatment, serum AST and ALT of two groups largely raised; and serum AST and ALT of the experimental group was much less than that of the control group (P<0.05, Table 3).

Table 3. *Comparison on serum AST and ALT of two groups before and after treatment.*

Group	n	Time	AST	ALT
Control group	48	Before treatment	98.32 ± 13.15	148.11 ± 23.12
		After treatment	73.57 ± 9.13*	98 ± 9.61*

| Experimental group | 48 | Before treatment | 99.21 ± 14.76 | 148.57 ± 22.22 |
| | | After treatment | 28.57 ± 6.59[*#] | 38.24 ± 8.61[*#] |

[*]P<0.05, compared with the same group before treatment, [#]P<0.05, compared with the control group after treatment.

Discussion

China is a moderate prevalent area of HBV infection, with a HBsAg prevalence rate of 7.18% in group aged from 1 to 59 y [5,6], and there are about 93 million chronic HBV carriers, of which 20 million are CHB patients [7]. The incidence of liver cirrhosis in the untreated CHB patients is approximate 2.1%, and among the patients infected HBV before six years old, about 25% will suffer from liver cirrhosis or HCC [4]. The annual incidence of decompensated cirrhosis induced by HBV or CHB is about 3%, and the cumulative incidence of 5 y is about 16%, and the 5 y case fatality rate of CHB, compensated or decompensated cirrhosis is 0%-2%, 14%-20%, and 70%-86%, respectively [4].

In recent years, with the increasing spread of HBV vaccination in China, the occurrence of CHB has been under control, but its morbidity and mortality are still on the rise, resulting in great suffering to patients and their families. The main manifestations of CHB in clinic include poor appetite, fatigue, abdominal bloating, nausea, hepatalgia and so forth [8], and the severe cases may have hepatosplenomegaly, jaundice, hepatalgia, fatty liver, liver cirrhosis, liver cancer and other serious complications, endangering the patients' lives. It is internationally recognized that IFN-α and nucleoside analogues are the main drugs for the anti-virus therapy of chronic hepatitis B. And IFN-α is the only drug approved for the anti-virus therapy of hepatitis B and immunoregulation that can stimulates the body to produce immune responses with HBV-specificity. Compared with nucleoside analogues, IFN-α cannot lead to drug-fast virus and has a relatively definite treatment course.

Accordingly, in the trial, we observe the changes of immune responses and pDCs functions in the patients before and after treatment and explore the effect of IFN-α on chronic hepatitis B patients and its mechanisms. The findings of the study reveal that peripheral CD4+ and CD4+/CD8+of the patients in both groups after treatment are much more than that before treatment (P<0.05). The increases of peripheral CD4+ and CD4+/CD8+ in the experimental group are more than that in the control group (P<0.05); compared before and after treatment, there is no difference in peripheral CD8+ of two groups (P>0.05). IFN-α is able to enhance patients' immune function. And T lymphocytes are a vital part of immune system that can reflect body immune condition. In CHB patients, CD4+ can promote B lymphocytes to produce antibodies, so as to clear away HBV [9,10]. Through inducing cells to synthetize anti-virus protein and restraining the duplication of HBV, IFN-α has a broad-spectrum antivirus effect. As a kind of natural antigen presenting cells, pDCscan synthetize and release a massive of IFN-α. A study demonstrates that the amount and functions of pDCs in CHB patients decline

[11,12]. The outcomes of the study indicate that after treatment, the serum IFN-α and peripheral pDCs of two groups go up largely; and compared two groups, the serum IFN-α and peripheral pDCs of the experimental group are more than those of the control group (P<0.05). IFN-α is able to improve the function of pDCs and rebuild the anti-virus effect of body. Therefore, serum AST and ALT levels of the patients in two groups after treatment are much higher than those before treatment; and that of the experimental group are much less than that of the control group (P<0.05), showing that the patients' liver function is regained.

In conclusion, we analyse the changes of serum AST, ALT, and IFN-α, and peripheral CD4+, CD8+, CD4+/CD8+, and pDCs of 96 CHB patients and confirm that IFN-α can largely enhance the immune responses and pDCs function of CHB patients and improve liver function, which provide some guides for clinical treatment and references for further studies on molecular mechanism.

Acknowledgements

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