Down-regulating CD4+CD25+T Cells Could Benefit CHB Patient with Mildly Elevated Alanine Aminotransferase by Applying Chinese Traditional Medicine

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Abstract

The hepatitis B virus (HBV) causes hepatitis B, an acute liver illness that raises the risk of liver failure, liver cancer, and cirrhosis. Chronic refers to a condition that lasts longer than six months. Currently, treatment is more biased toward severe patients. However, the therapy for patients with elevated ALT is also necessary but needs to be addressed. This paper will explore how the Chinese Traditional Medicine Bushen formula could benefit CHB patients will elevate ALT by reducing the number of CD4+CD25+T Cells. This work will use Flourescence-Activated Cell Sorter (FACS) and real-time PCR to measure the percentage of T-reg cells in mice with elevated ALT levels after treatment with the Bushen formula. The effectiveness of the Bushen formula on CHB patients has been proved in this paper, which could help millions of patients to have a better life quality once the side effects and the dosage for each individual are explored further.

Keywords: ***CHB, Chinese Traditional medicine, ALT, CD4+CD25+T Cells, Immune system***

1. Introduction

Hepatitis B virus (HBV) infection is a pervasive and serious public health issue, especially in China. Around 350 million people around the world have HBV infection and become carriers. Every year, complications connected to HBV are responsible for more than 750000 deaths. In addition to being more likely to develop cirrhosis and decompensation, HBV carriers are 100 times more likely to develop hepatocellular carcinoma (HCC) [1,2]. To decrease the prevalence and mortality of chronic hepatitis B (CHB) infection, early identification and treatment are thus imperative [3].

Currently, there are two diverse treatment methods for CHB patients: uses immunomodulators such as standard or pegylated interferon-α, also long-term treatments with the nucleostide analogues lamivudine [4]. However, these treatments are only recommended for most individuals with an elevated ALT. On the other hand, mildly elevated ALT patients also need to be treated properly.

The New research shows that the Chinese Traditional Medicine Bushen formula could have a significant effect in this case by down-regulating CD4+CD25+T cells. These naturally occurring Treg cells are appears in the periphery and are able to inhibit proliferation and affects T cells in vitro and in vivo [1]. This paper will discuss about whether decreasing the percentage of CD4+CD25+T cells has a positive effect on CHB patients due to the relevant immune mechanism. When persons with chronic HBV infection, CD4+CD25+T cells may be reallocated to the liver because of their immunosuppressive function, where they may create a long-lasting low-level inflammatory state [2]. I believe that bushen therapy, which lowers the fraction of CD4+CD25+T cells, will be beneficial for the CHB mouse patient who has slightly raised ALT. Bushen is administered to CHB mice at progressively higher doses for varying lengths of time. Treg abundance is then determined by FACS for CD4/CD25/FOXP3 and HBV levels are determined by PCR. Count the number of cytotoxic CD8/CD45/cd54 T cells as well using FACS. Therefore, it may be appropriate to reduce the CD4+CD25+T cell activity.

2. Material and methods

2.1 *Animal Models and Groups*

The experiments will use CHB mice with mildly elevated ALT and lamivudine which is a common nucleostide analogues used to treat HBV. The mice will be divided into 3 groups: (1) negative control: CHB mice without any treatments; (2) positive control: CHB mice treated with lamivudine; (3) experiment group: CHB mice treated with Bushen formula.

2.2 *Flourescence-Activated Cell Sorter (FACS)*

FACS is used to detect the ratio of CD4+CD25+ T cells and CD8+ T cells.

Phosphate buffered saline containing 1% sodium azide and 0.02% BSA was used to wash the PBMC twice. By pre-incubating cells from the supernatant of the hybridoma cell line 2.4G2, non-specific antibodies binding to Fc

receptors were prevented. After being incubated with the appropriate mAb (0.5 g/106 cells) for 30 min at 4°C, the cells were twice washed. Using the BD LSR II and CELL Quest software, multicolored flow cytometry analysis was carried out (Becton Dickinson immunocytograph system). The aforementioned chemicals and mAbs were purchased: Anti-CD4 mAb GK1.5 is combined with fluorescein-isothiocyanate (FITC) and erythrotin (PE), anti-CD8 mAb 53-6.7 is combined with FITC-, PE and isoisocyanin (APC), and anti-CD25 mAb 7D4 is conjugated with APC. For the purpose of identifying HBV-specific CD8+ T cells, DimerX recombinant soluble dimer mouse H-2LD Ig was utilized. To load S28–39 peptides onto DimerX, follow the manufacturer's instructions. The S28-39 peptide and anti-CD8 mAb were incubated in DimerX at 4°C for 2 hours before being thoroughly cleaned. Using multicolor flow cytometry, PE in combination with anti-mouse IgG1 mAb A85-1 mAb was incubated at 4°C for 25 min [5].

CD4+ CD25+ cells (2 106 to 3 106/well) were stimulated with rpMI-10% FCS of 1 mM of different peptides in order to deplete total PBMC or PBMC. Recombinant IL-2 was added on day four of culture, and 10–12 days later, the frequency and functioning of CD8+ cells were assessed. CD4+ CD25+ cells were re-added to PBMC that had been depleted of CD4+ CD25+ in many trials, with a responder/regulator ratio of 20:1 [6].

2.3 Real-time PCR

A 50 µl reaction mixture, including the TaqMan universal PCR Master Mix, 20 mM primer, and 20 mM probe, was added for amplification, and 5 µl of DNA was extracted. Every sample is taken twice. As our internal check, we used a TaqMan exogenous internal positive control kit that included 1X IPC Mix, 1X IPC DNA, VIC-labeled primers, and TaqMan probes. Every agent is a product of Applied Biosystems.

ABI PRISM 7500 Real Time was used to perform absolute measurement of HBV DNA. Uracil N'-glycosylase was first inactivated by incubating the probable contaminant amplicon at 50°C for 2 min, followed by activation of the AmpliTaq gold polymerase and uracil N'-glycosylase at 95°C for 10 min. The 45 two-step PCR cycle are 95°C for 20 s and 60°C for 60s. (general condition).

Before performing linear testing on the D and F genotypes, two international HBV DNA qPCR tests created by BBI Diagnostics were validated first: PHD801 (diluted 1-500000x at 4.3 106 IU/mL, genotypes A) and QHD701 (diluted 1.19 106 IU/mL diluted 1-15000x, genotypes A).

Additionally, Versant HBV DNA Test (n = 58) findings were compared with QPCR results. Real-time PCR was employed in this investigation to identify the precore/core region of the HBV genome. Its linear range is from 102 to 5 X 109 IU/mL.

Genotypes A (7.25 log IU/mL) and C (7.40 log IU/mL) from two clinical samples were utilized. Using samples of the three most common genotypes (20, 50, 100, and 150 IU/mL), the limits of detection were determined [7].

3. Possible results

Table 1. Combination of possible results(CR)

Possible observations	CR1	CR2	CR3	CR4	CR5	CR6
CD4+CD25+FoxP + T cells decreases?	+	+	+	+	-	-
CD8+T cells increases?	+	+	-	-	-	-
HBV DNA decreases?	+	-	+	-	+	-
Supporting Hypothesis?	YES	Partially	Partially	Partially	Partially	NO

Note. "+" represents a positive result. "-" represents a negative result.

Possible Result 1: Applying Bushen formula regulates Treg cells and HBV DNA level decrease

Table 1 shows that the Bushen formula could down-regulating CD4+CD25+ T cells and up-regulating CD8+ T cells which lead to the decreasing of HBV DNA level. The animal experiments display that Bushen formula can benefit CHB patients.

Possible Result 2: Applying Bushen formula can regulates Treg cells but HBV DNA level does not decrease

As shown in table one, Bushen formula is able to up-regulating CD8+ T cells and down-regulating CD4+CD25+ T cells. However, the HBV DNA level does not decrease. The animal experiments display that Bushen formula does not have a significant effect on CHB patients.

Possible Result 3: Applying Bushen formula can down-regulating CD4+CD25+T cells only and HBV DNA level decreases.

Bushen formula can down-regulating CD4+CD25+ T cells only but has on positive effect on CD8+ T cells. The animal experiments display that Bushen formula can only partially benefit CHB patients (see table 1).

Possible Result 4: Applying Bushen formula can down-regulating CD4+CD25+T cells but cannot decrease HBV DBA level.

Table 1 demonstrates Bushen formula can down-regulating CD4+CD25+ T cells only but has no positive effect on CD8+ T cells and the HBV DNA does not decrease. The animal experiments display that Bushen formula cannot benefit CHB patients.

Possible Result 5: Applying Bushen formula does not regulate Treg cells but HBV DNA level decreases.

Bushen formula has no effect on either CD4+CD25+ T cells or CD8+ T cells. However, HBV DNA level decreases. Since the experiments are using mice model The animal experiments display that Bushen formula cannot

Possible Result 6: Applying Bushen formula does not regulate Treg cells and cannot decrease HBV DNA level.

Bushen formula has no effects on Treg cells and there is no change in HBV DNA level. The animal experiments display that Bushen formula will not benefit CHB patients (see table 1).

4. Discussion

Bushen formula can be used as an alternative treatment with confidence, according to the most recent recommendations, which specify that the aim of CHB treatment is to enhance quality of life and survival by halting the progression of the illness to cirrhosis, decompensated cirrhosis, end-stage liver disease, liver cancer, and death [7,8].

Bushen formula, which was used to energize the spleen, tonify the kidney, and clear dampness, is made up of Astragalus mongholicus, Fructus Ligustri Lucidi and Longspur Epimedium [9]. Components of the Bushen formula have been demonstrated to have broad-spectrum immunomodulatory effects. In burning mice infected with Pseudomonas aeruginosa, astragalus mongholicus may reduce Treg activity by binding to TLR4 on Treg and initiate th2-th1 transfer by activating CD4+ T cells [10]. By obtaining more patient samples for future research, we will deepen our understanding of the immunomodulatory mechanisms of BSF in CHB patients.

Possible result 1 fully supports my hypothesis that Bushen formula can benefit CHB patients by down-regulating CD4+CD25+T cells and up-regulating CD8+ T cells. For further investigations, the side effects of Bushen formula should be tested to make sure its security.

Possible result 2 illustrates the fact that Bushen formula can regulate Treg cells. The reason why HBV DNA level does not decrease maybe is duration of the treatment does not last for the enough time since Chinese Traditional Medicine is relatively gentle to patients and takes longer. Furter investigations should explore a suitable treatment duration of Bushen formula.

Possible results 3 and 4 both prove the Bushen formula can down-regulating the CD4+CD25+ T cells, but no effects on CD8+ T cells. However, possible result 3 has a decreasing on HBV DNA level but possible result 4 has not. It might be associated to the immune system condition of different individuals. Based on this, further investigation should pay attention to the different requirement of individuals and make scales with different amount/concentration of medicine.

Possible results 5 and 6 can be similar to passible results 3 and 4. The only difference is the ratio of CD4+CD25+ T cells. Bushen formula does not demonstrate any function on regulate Treg cells. The decreasing of HBV DNA level in result 5 may be caused by an individual's immune system or an error occurs during the experiments.

Unlike western medicines, the Chinses Traditional Medicine have a relatively gentle therapeutic effects which means they might use longer time to reach it possible consequences. CHB is a chronic and sever liver disease and more western treatments are used for patients with high ALT level. However, for CHB patients with mildly elevated ALT, the duration and intensity of treatment can be reduced and Chinese Traditional Medicine seems more suitable. Although, the security of Chinese Traditional medicine has not been tested, but this is an area where research should be expanded to see if it can benefit CHB patients to a greater extent.

5. Conclusion

Bushen formula from Chinese Traditional Medicine demonstrated better clinical efficacy against CHB. Fox3 expression and the frequency of CD4+CD25+T cells would both significantly rise in CHB patients with mildly raised ALT levels. They would benefit from using the Bushen formula from Chinese Traditional Medicine since it may lower the serum ALT and HBV DNA levels by reducing the number of CD4+CD25+T cells and raising the fraction of CD8+ T cells. As a result, for CHB patients with minimally raised ALT, the Bushen formula may be was identified as the best therapeutic strategy. However, more randomized controlled trials of high quality are needed to provide more reliable clinical basis for the application of TCM. Further research should be done on the side effects of Bushen formula, the length of the therapy, and the best dose for each individual.

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