

Astragalus Mongolians' Suppression of HBV DNA Level in Chronic Hepatitis B (CHB) Patients with Moderately Increased Alanine Aminotransferase (ALT)

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Abstract

This paper aims to examine the impact of Astragalus on the quantity of HBV DNA in chronic hepatitis B (CHB) patients with slightly raised ALT and the effect on IFN-G. Moreover, the work predicted that IFN-gamma would increase after taking Astragalus Mongolians in CHB mouse patients with mildly raised ALT and effectively suppress the amount of HBV DNA. Three groups of mice were set up, including a positive control group, a negative control group, and a group of mice with mildly elevated ALT, which are more significant than one to two times the upper limit of normal (1-2 times ULN) and carrying CHB. Astragalus was administered to mice previously infected with HBV in increasing doses and for longer lengths of time. Entecavir was a positive control, while PBS + DMSO was the negative control. IFN-g in the blood was detected using an ELISA test, and HBV DNA was determined using RT-PCR. The six possible results in this paper correspond to the reaction of CHB-carrying mice with mildly elevated ALT to Astragalus. Whether the amount of IFN-G and HBV DNA was elevated or reduced. The results of this paper will effectively predict the effect of Astragalus on the amount of HBV DNA in CHB-carrying mice with mildly raised ALT with several different outcomes. This paper also analyzes the different outcomes and compares the changes in IFN-G; corresponding conclusions will be drawn based on the different outcomes.

Keywords: -IFN-G, ALT, HBV DNA, Chronic hepatitis B, Astragalus Mongolians

1. Introduction

Hepatitis B, which is further classified into acute and chronic forms, is an infection of the liver brought on by exposure to the hepatitis B virus. 3,322 instances of acute hepatitis B were reported in the US in 2018, and the CDC estimates that 862,000 Americans (almost half the population of Idaho) have chronic hepatitis B. Hepatitis B is present in 257 million people worldwide [1]. Additionally, the HBV virus raises the chance of liver cancer, which finally arises due to cirrhosis. Alanine aminotransferase, also known as ALT, is mostly present in liver cells and is released into the blood when those cells are harmed. HBV infection is one of the main reasons for high ALT. The frequency of severe histological illness increases with age and ALT levels in people with modestly high ALT levels [2]. Astragalus is an effective medicinal herb in traditional Chinese medicine. It includes several active components, including polysaccharides, saponins, and flavonoids, which play crucial roles in hepatoprotection, antioxidant activity, immunological activation, and antiviral activity [3]. The effects of astragalus on CHB patients with modestly raised ALT levels will be covered in this paper, with particular attention paid to changes in HBV DNA levels and changes

in IFN-g expression levels.

2. Methods and Materials

The RT-PCR technique will be used to calculate the amount of HBV DNA present in this experiment by initially taking samples from each of the three groups of mice. RT-PCR is used to quantify HBV DNA after that. Using the SMI Test EX-RandD (Sumitomo Metal Industries, Tokyo, Japan) in accordance with the manufacturer's instructions, total DNA was extracted from 100 µl of serum. 20 µl of distilled water was used to resuspend the purified DNA. For RT-PCR, 10 µl of DNA solution (equal to 50 µl of serum) was utilized. RT-PCR was carried out using an ABI 7700 sequence detection device and a PCR core kit (Perkin Elmer, Foster City, Calif.). The amplification reaction mixture (50 µl) contains 200 m dATP, 10 µl DNA solution, 5 µl 10 Taq Man buffer A, 200 µm dCTP, 200 µm dATP, and 500 µm dUTP, 200 µm dGTP, 3. 5 mM MgCl₂, 200 nM reverse primer, 200 nM forward primer, 300 nM Taq Man inquiry 0.5 U of Amp Erase uracil N-glycosylase and 1.25 U of Ampli Taq Gold DNA polymerase (UNG). Conditions for thermal cycling were as follows: The first 2 minutes of UNG activation at 50°C were followed by Taq Gold activation and 10 minutes of UNG inactivation at 95°C.

Then, 53 cycles of amplification at 95°C for 20 seconds and 60°C for 1 minute were completed. RT-PCR was used to measure HBV DNA in a blinded test—serology and bDNA hybridization testing. By signal amplification with enzyme-labeled bDNA (Quantiplex HBV DNA; Chiron Corporation, Emeryville, CA), HBV DNA in serum was measured. Follow the instructions provided by the manufacturer. For Quantiplex HBV DNA, the limit of quantification was roughly 7×10^5 DNA copies/ml [4].

The level of IFN-G in mouse blood was measured using an ELISA test using a commercially available kit (ThermoFisher Scientific). The ELISA is used to quantify how much target is bound between matched pairs of antibodies. Overnight, target-specific antibodies are added to the bottom of the wells of a microtiter plate. Once in these wells, a sample, standard, or control is added and binds to the immobilized (captured) antibody. The enzyme-antibody-target complex combines with the substrate solution to create a quantifiable product, and a second (detector) antibody is added to form an intercalation layer. The concentration of the target in the original specimen directly relates to the strength of this signal [5].

2.1 Animal Model

The experiment divided the mice into three groups: the positive control was treated with entecavir; the negative control was vehicle (PBS+DMSO); the experimental group was treated with astragalus 3 grams per day and treat for one week.

3. Result

There are six possible results.

Possible Result 1: After taking astragalus, CHB-carrying mice with modestly raised ALT levels exhibited lower levels of HBV DNA than negative and positive controls and higher blood IFN-G levels.

The study shows that astragalus is superior to entecavir and the negative control group and has a very effective inhibitory effect on HBV DNA in CHB-carrying mice with modestly raised ALT. A negative regulator is IFN-G. As a result, it fully confirms the hypothesis.

Possible Result 2: After taking astragalus, mice with CHB and modestly high ALT levels had lower levels of HBV DNA than both negative and positive controls, and their blood levels of IFN-G were lower.

The study shows that astragalus is superior to entecavir, has a very good inhibitory effect on HBV DNA in CHB-carrying mice with modestly high ALT levels. IFN-G, however, dropped. As a result, the evidence does not

support the hypothesis.

Possible Result 3: The mice with CHB and modestly raised ALT levels had lower levels of HBV DNA than the mice in the negative control group but higher levels than the mice in the positive control group, and their blood levels of IFN-G were higher.

Given that their HBV DNA values are lower than those of the negative controls in CHB-bearing mice with modestly raised ALT, the results of this experiment imply that astragalus has an inhibitory impact on HBV DNA. At the same time, its effect is weaker than that of entecavir. A negative regulator is IFN-G. The hypothesis is thus only partially supported.

Possible Result 4: IFN-G levels dropped and the amount of HBV DNA in the blood was smaller in CHB-bearing mice with modestly elevated ALT than in the negative control group but bigger than in the positive control group.

Considering that the HBV DNA values of the CHB-bearing mice with mildly raised ALT are lower than those of the negative controls and that Astragalus' effect is milder than that of entecavir, the results of this experiment imply that Astragalus has an inhibitory effect on HBV DNA. IFN-G functions as a positive regulator. The hypothesis cannot be proven; hence it is unsupported.

Possible Result 5: The amount of HBV DNA in CHB-bearing mice with modestly raised ALT was higher than that in the negative control group and greater than that in the positive control group, and the amount of IFN-G in the blood was increased.

The study shows that in CHB-carrying mice with mildly increased ALT, astragalus had no inhibitory effect on HBV DNA. They had higher HBV DNA readings than the negative control group, which implies that astragalus had the opposite impact. IFN-G functions as a positive regulator. As a result, the evidence does not support the hypothesis.

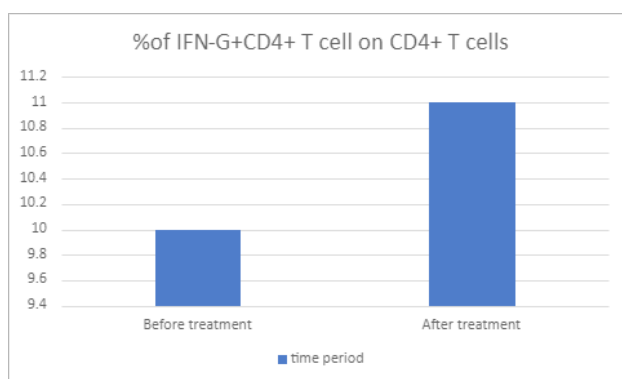
Possible Result 6: IFN-G levels in the blood were decreased, and the amount of HBV DNA in CHB-bearing mice with modestly raised ALT was higher than that in the negative control group and greater than that in the positive control group.

The study shows that in CHB-carrying mice with mildly increased ALT, astragalus had no inhibitory effect on HBV DNA. They had higher HBV DNA readings than the negative control group, which implies that astragalus had the opposite impact. IFN-G functions as a negative regulator. As a result, the evidence does not support the hypothesis.

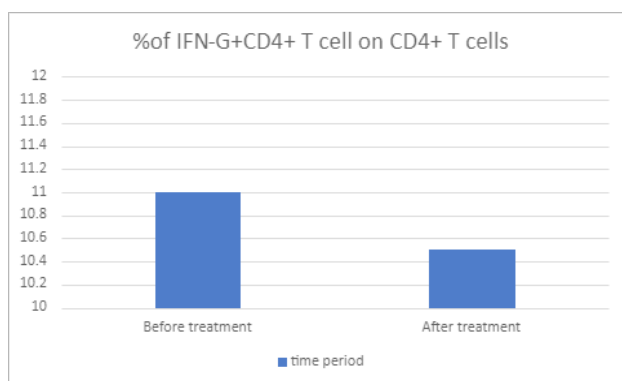
Table 1. Possible Results

Cell Lines	Assay	Possible Result 1	Possible Result 2	Possible Result 3	Possible Result 4	Possible Result 5	Possible Result 6
<i>In Vivo Model</i>	IFN-G increases	+	-	+	-	+	-
	HBV DNA decreases	++	++	+	+	-	-
	Support the hypothesis?	Totally support	Do not support	Partially support	Do not support	Do not support	Do not support

Note. For the level of IFN-G, “+” represents a significant increase, “-” represent a significant decrease. For HBV DNA, “++” represents HBV DNA count less than positive control group, “+” represents HBV DNA count less than positive control group, but greater than negative control group, “-” represents HBV DNA count less than positive and negative control group.



(A)



(B)

Figure 1. Two possible results in comparing the percentage of IFN-G + CD4+ T cell on CD4+ T cells after the Astragals treatment.

4. Discussion

The reduction in viral load, serum ALT levels, and TCM symptom composite scores following the Bushen formula support the findings of earlier studies, which

found that the Bushen formula benefits CHB patients with modestly increased ALT, as shown in Table 1. When CHB patients with modestly raised ALT are turned away from therapy due to the current ALT-dependent treatment initiation criteria, the Bushen formula offers an excellent alternative. The paper proposed that the frequency of CD4+CD25+ T cells is correlated with these advantageous outcomes. In line with this theory, scientists discovered that the frequency of CD4+CD25+ T cells was inversely correlated with the degree of IFN-g expression in CD4+ T cells and that after Bushen formula administration, the frequency of CD4+CD25+ T cells decreased and the degree of IFN-g expression in CD4+ T cells rose [6]. As shown in Figure 1, (A) matches the possible results 1, 3, and 5. (B) matches with possible results 2, 4, and 6. Therefore, astragalus stem and leaf flavonoids (FAM) can promote ConA-induced lymphocyte proliferation, increase T cell numbers and regulate T cell subpopulation abnormalities, and elevate IL-2-induced LAK activity since astragalus is a part of the Bushen formula [7]. So possible results 1 and 3 are most likely to be presented.

5. Conclusion

With the widespread use of traditional Chinese herbal medicine worldwide, the research in this paper is valuable. The modulatory effect of IFN-G on HBV DNA was also covered in this study's discussion of whether Astragalus inhibits the amount of HBV DNA in CHB-carrying mice with modestly increased ALT. Future studies should also investigate the pathways through which the active ingredients in Astragalus inhibit the virus, which chemical components of Astragalus play a significant role, and for which area of the immune system they function to provide the framework for the transition to clinical trials.

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