

The Comparison between HBV Transgenic Mice and Normal Mice after Being Injected Concanavalin A (Con A)

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Abstract

HBV transgenic mice are used as chronic hepatitis B models for research, but they cannot be infected by HBV to have the hepatitis B as the same as human. In the research, we explored the differences between HBV transgenic mice and normal mice who are injected the concanavalin, A which can injure the livers of the mice. We found some data to show the differences of them after the injection, such as weight, pictures of the liver tissue, alanine transaminase (ALT) and aspartate transaminase (AST) in the blood. The pro-inflammatory cytokines (IL-6, IFN- γ and TNF- α) and T cells (CD4⁺ cells and CD8⁺ cells) also showed the livers of HBV transgenic mice were more vulnerable to be hurt than normal mice.

Keywords transgenic mice; Concanavalin A; Hepatitis B

Introduction

Nowadays, chronic hepatitis B, as a major hidden danger [1], is a threaten to human being's health, but there is a lack of effective measures and radical solutions to cure it [2,3]. This situation has an inseparable relationship with the persistent infection of HBV and the relative lag of the research of pathogenic molecular mechanism and the development and evaluation of antiviral drug, on account of the short of the susceptible cell models and animal models to HBV [4,5]. Therefore, establishing an effective model like HBV infectious lab animals or cells is crucial to the development of effective antiviral drugs and vaccines [6].

Now, HBV transgenic mice are gradually used for experiment and research about hepatitis B. HBV transgenic mice are this kind of mice which can be used for research: At first, injecting the whole HBV genome or one fragment which has been purified and modified in advanced into the male pronucleus of the mice cell fertilized eggs, then implanting the fertilized eggs into the receptor female mice. After a normal period of gestation, if the newborn mice are identified they can copy, transcribe and express the HBV gene, when they grow up; they are gathered to mate with the normal mice who have the same background as them to build pure line. In the offspring, the mice who are able to copy, transcribe and express the gene of HBV are called HBV transgenic mice [7].

HBV transgenic mice carry the hepatitis B virus, but they cannot be persistently infected by HBV [8]. According to the literatures [9-11], injecting concanavalin A to the caudal vein of mice can cause the chronic injury to their livers, which will contribute to establish a model of chronic hepatitis B for research. What we found in the experimental process is that concanavalin A could incur different effect correspondingly to the HBV transgenic mice and normal mice. The Con A-caused damage to the livers of HBV transgenic mice is greater than that of normal mice, showing that compared to normal mice, HBV transgenic mice have weaker tolerance capacity to concanavalin A. In this way, maybe we can explore the infecting mechanism of HBV. In this paper, we will elaborate this phenomenon in detail.

Materials and Methods

Mice and grouping

All mice used for experiment were female BALB/c mice, at the

age of six to eight weeks, weigh above 20 g, in SPF level. Normal mice were obtained from Laboratory Animal Center of Southern Medical University (China) and Medical Laboratory Animal Center of Guangdong (China). And the HBV transgenic mice were built and bred by 458 Hospital. All the mice were housed in the animal facilities of 458 Hospital with free access to food and water. They were kept in an air-conditioned room at $23 \pm 2^\circ\text{C}$ with a 12/12-h light and dark cycle. Before experiment, all the animals were allowed to acclimate for at least 1 week. There were two experimental groups as HBV transgenic mice group and normal mice group with 10 mice in every group respectively.

Concanavalin A injection

Concanavalin A (CAS Number: 11028-71-0, purchased from Sigma-Aldrich Corporation) dissolving in 0.9% saline was injected via caudal vein at 8 mg/kg body mass in two groups. The weights of mice were recorded before and after the injection. All procedures were performed in accordance with the Ethics Review Committee for Animal Experimentation of Institute of Clinical Pharmacology, 458 Hospital.

Evaluation of liver injury

The mice blood was collected from the retro-orbital sinus 16 h after Concanavalin A administration. The collected blood samples were kept at 37°C calortat for 90 min, centrifuged at 10,000 g for 10 min, and the separated sera was stored at -20°C until analysis. BS-480 Chemistry Analyzer (Mindray, China) measured activities of the alanine aminotransferase (ALT) and the aspartate aminotransferase (AST) in serum.

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Histological analysis

16 h after the Con A treatment, all the mice were killed, and each liver was collected. Livers were soaked in 4% paraformaldehyde for 2 days, dehydrated in a graded series of ethanol and xylene, and embedded in paraffin. Thin sections were stained with hematoxylin and eosin (H&E).

Flow cytometric analysis

The collected livers were passed 200 mesh metal sieve after being perfused and cut up, then diluting the cell suspension to 6 ml. According to the instruction, the percoll was diluted to 33%, and getting 3 ml the diluted solution to 15 ml centrifuge tubes. Then the cell suspension from mice livers were slowly added to the cell separation fluid. At 25°C, with both of raising speed and reduction of speed of 1, the solution was centrifuged at 450 g for 15 min. The precipitation in the bottom was liver lymphocytes mixing with a certain amount of erythrocyte and liver parenchyma cells. After discarding the upper liver tissue mass and other residue, the erythrocyte was splitted according to the manual, and after being centrifuged, the lymphocytes were collected. In order to identify lymphocytes, mononuclear cells were stained by PE-labeled anti-CD4 Ab (BD Biosciences, USA) and FITC-labeled CD8a Ab (BD Biosciences, USA). The counts of infiltrating CD4+ cells and CD8+ cells in the liver were analyzed by BD FACS Calibur (BD Biosciences, USA).

Inflammatory factor analysis

16 h later since finished Con A injection, the collected blood of experimental mice was used for testing the total levels of tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ) and interleukin-6 (IL-6) by using a commercially available ELISA kit (purchased from Multi sciences) based on the manufacturer's recommendations.

Statistical analysis

The data expressed is the mean \pm standard deviation and statistical analysis was carried out by Prism 6.0 (Graph Pad Software) and spss 19.0. One-way analysis of variance was used to compare all difference among both of groups. For multi-group analysis, intergroup imparity was compared by Dunn's test. $P < 0.05$ was considered to indicate significantly different.

Results

The difference of ALT and AST

In one week after injecting the Con A, the blood of the transgenic mice and normal mice were collected with the passage of time. The collected blood was used for measure ALT and AST, and got the contrast hyperbolic figure of ALT and AST (Figures 1A and 1B). As we know, if content of ALT and AST in the blood grow up, we can say that the livers are injured badly. For HBV transgenesis mice, both of ALT and AST rose sharply in 6 h after the injection of concanavalin A and peaked at the 14th h. Well these of the mice in normal group rose up at the 8th h and reached the highest point at the 16th. And the highest of the HBV group was almost 4 times than the normal group's. What's more, the ALT and AST of HBV group mice were higher than the normal mice, and continued to be much higher until the fourth day when the two groups were brought into correspondence with each other. Thus we find that the damage of Con A in transgenic mice is more sensitive than in normal mice.

The comparison of weight

After a week of injecting the Con A, we got the mice's weights, and after getting the average weight we discovered that the mean of the HBV transgenic mice declined by 5.82% and the normal declined by 3.22% (Figure 1C).

Histology

During the experiment, at the different time points after the injection, the mice were killed and the livers were collected. After the liver was stained with hematoxylin and eosin, we compared and got some differences between the liver tissues of HBV transgenic mice and the normal mice. The hair of HBV transgenic mice adhered to the body without burnish at the 16th h after the injection of concanavalin A injection (Figure 1D). The hair also lost the gloss in the normal mice group at the same time, but it is lighter than the circumstance of HBV transgenic mice group (Figure 1E). And the livers of HBV transgenic mice after the injection had obvious boundary of necrosis (Figure 1F).

The difference of CD4+ and CD8+

It was a striking evidence indicating that T cells had played a critical role in Concanavalin A-induced liver injury (Figures 2A-2F) [12,13]. So we show the change of T cells by calculating the percentage of CD4+ and CD8+ cells in the liver which used the flow cytometry (Figures 3A and 3B) [14-16]. As expected, the percentage of CD4+ and CD8+ were different between the two groups after Concanavalin A injection. The percentage of CD4+ and CD8+ of the HBV transgenic mice were much higher than these of the normal group. These data indicate that Concanavalin A could hurt the HBV transgenic mice more seriously than the normal mice (Figures 3C and 3D).

Pro-inflammatory cytokines

According to the literatures, the accumulating evidences demonstrated that Concanavalin A-induced liver injury can be mediated by pro-inflammatory cytokines, such as IFN- γ , IL-6 [17], and TNF- α . In our study, IFN- γ , IL-6, and TNF- α in serum were separately measured by ELISA [15,18,19]. 16 h later, after the Con A intravenous infection, we found these pro-inflammatory cytokines were significantly increased in serum with the level of IFN- γ , IL-6, and TNF- α of the normal mice who were not injected Concanavalin A was in the zero level. By these data, we can see that Concanavalin A could induce the level of IL-6 and IFN- γ rising up except TNF- α (Figure 4). And for IL-6, the HBV transgenic mice were hurt more badly compared the normal group.

Discussion

Injecting Con A via caudal vein to build acute hepatitis B model has been reported in many literature [20,21], injection of a certain concentration of Concanavalin A can cause certain damage to liver of mice, and if it for a long time there will be a possibility of liver fibrosis [22]. From the literatures before [14], we knew that 8 mg/kg was the lowest concentration of Con A which could cause the livers injured. In the long-term experiments, we found that if we injected the Concanavalin A at 8 mg/kg, the normal mice and HBV transgenic mice would bear the different degrees of damage at the same living conditions [9-11,16].

The experiment displayed the degree of the damage to the livers of the mice by detecting the concentration of the transaminase, such as ALT and AST in the blood which was collected at the time point after the injection of the concanavalin A. The result of the detection

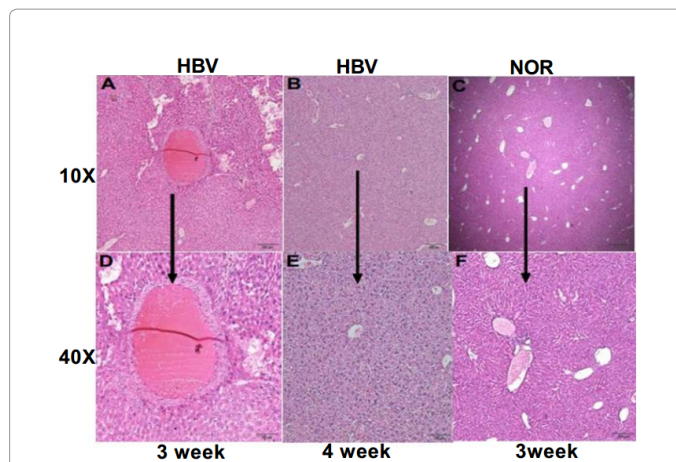
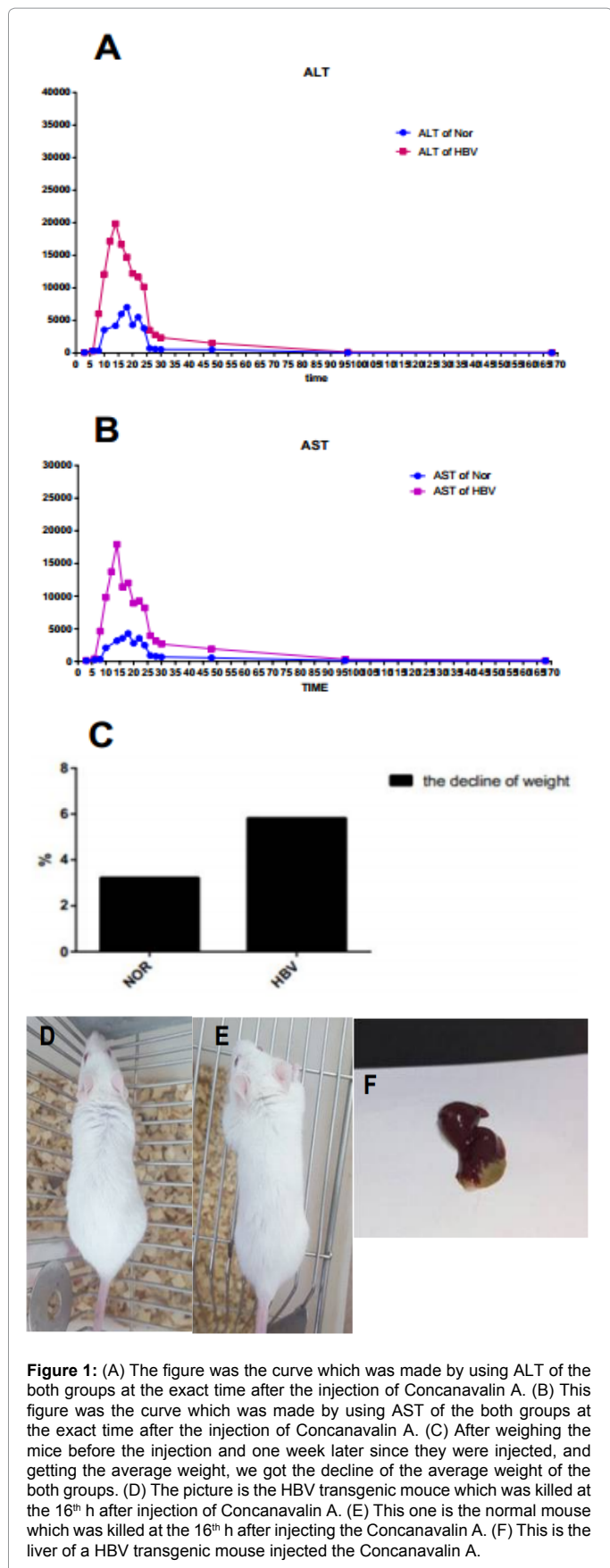


Figure 2: The liver tissue slices of the mice. (A and D) The liver of HBV transgenic mice who were injected Con A onetime every week for three weeks. (B and E) The liver of HBV transgenic mice who were injected Con A onetime every week for four weeks. (C and F) The liver of normal mice who were injected Con A onetime every week for three weeks.

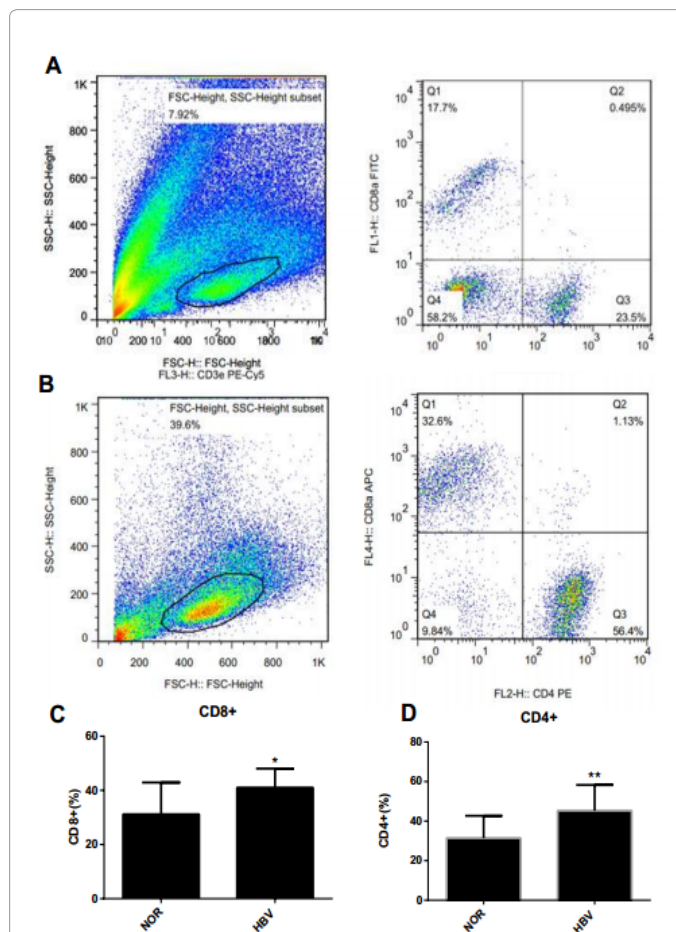
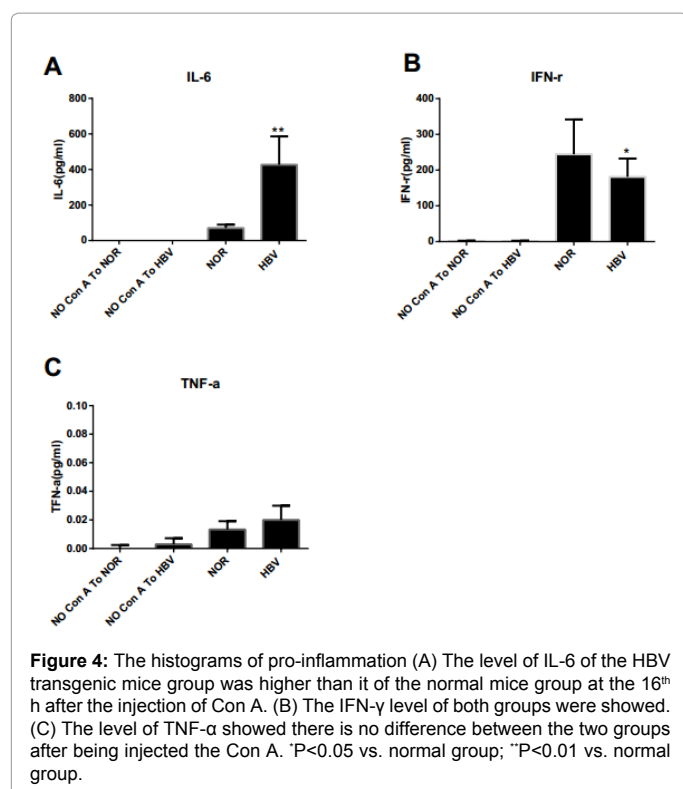


Figure 3: (A) Representative images of CD4⁺ T cells and CD8⁺ T cells from the normal mice group are shown. (B) The images of CD4⁺ T cells and CD8⁺ T cells from the HBV transgenic mice group are shown. (C) The image shows the percentage of CD8⁺ of the normal mice group and HBV transgenic mice group. (D) The image shows the percentage of CD4⁺ of the normal mice group and HBV transgenic mice group. *P<0.05 vs. normal group; **P<0.01 vs. normal group.



showed us that the ALT and AST of the HBV transgenic mice began to rise at the 6th h after injection, while the normal mice's ALT and AST got to rise at the 8th h, implying that under the same concentration of concanavalin A, the livers of the HBV transgenic mice were more vulnerable. The ALT and AST of the HBV transgenic mice reached the peak after 14 h of the injection and the normal mice reached to the peak at the 18th h, and the peak of the HBV transgenic mice was four times as higher as that of the normal mice. At the same time, we weighed the mice before the blood sampling, what we find is that the average weight of the HBV transgenic mice fell off more than of the normal mice. This phenomenon told us that the livers of the HBV transgenic mice are more vulnerable and at the same time the severity of injury is higher than those of the normal mice.

After 16 h of injecting the Con A, the mice of two groups had gotten some different changes about body surface characteristics. The HBV transgenic mice's hair lost luster and adhered each other, the mice moved slowly and weakly, while for the normal mice, the hair was lack of luster likewise, and they are more quick-witted than the HBV transgenic mice. At the different points of time after the injection we killed the mice and collected the liver tissues of mice, and observed the specimens after being stained with hematoxylin and eosin, at the end we found that, the clearance of liver cells from HBV transgenic mice had largened, and it was a severer case than the normal mice.

T cells in the livers can be used as the standard of damage for livers [23]; in this experiment, we detected the level of CD4⁺ cells and CD8⁺ cells by means of the flow cytometry detection. Comparing the two groups of mice, we could find that in 16 h after the injection of Concanavalin A, the concentration of the CD4⁺ and CD8⁺ in HBV transgenic mice livers is higher than that in the normal mice's livers, which told us that the HBV transgenic mice were more facile to be damaged by the stimulation than the normal mice in immunology.

If the level of IL-6, IFN- γ and TNF- α have a change, we can say that the balance of the health for the liver of the mice has been damaged [24]. From the result of ELISA, we can see that the level of pro-inflammatory cytokines has different degrees of changes, which indicates that the livers of the mice in the two groups has experienced some damage and they suffered the injury in varying degrees.

Above all, we got some data to see the differences between the two groups after being injected the Con A. In conclusion, the HBV transgenic mice are easier to be hurt and the severity of injury is worse than the normal group, implying the gene of the hepatitis B virus in the mice could conduce some unknown influence on the livers. Although HBV transgenic mice could not be injected by HBV to have hepatitis now, but if we can explore the mechanism for further study, maybe we can get some progress to establish a model for chronic hepatitis B. The result would promote the development of the animal models of the HBV for the future.

Acknowledgments

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