Effect of HBIG combined with hepatitis B vaccine on blocking HBV transmission between mother and infant and its effect on immune cells

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Abstract. The effect of hepatitis B immune globulin (HBIG) combined with hepatitis B vaccine on blocking hepatitis B virus (HBV) transmission between mother and infant and its effect on immune cells were studied. Ninety newborn infants confirmed to be HBV surface antigen (HBsAg)-positive were divided equally into three groups. Group A newborns received the hepatitis B vaccine at 0, 1 and 6 months after birth (10 μg/time). Group B newborns received an intramuscular injection of 100 IU HBIG 2 h after birth before the same treatment as group A. Mothers of group C newborns received three gluteus maxinus injections of 200 IU HBIG. The newborns in group C got the same treatment as group B. The blocking effect of HBV transmission between mother and infant was evaluated, and cell immune function was assessed. There were significant differences in comparison of blocking success rates between group A and B, and between group A and C as well ($p<0.05$). At the end of 12 months follow-up, the $CD4^+$ level and $CD4^+/CD8^+$ ratio in group C were higher thanthose in group A and B ($p<0.05$). In addition, the level of $CD8^+$ T lymphocyte in group C was lower than those in group A and B ($p<0.05$). In comparison of levels of $CD4^+$T lymphocyte at the end of 12 months follow-up and 24 h after birth, the differences were significant ($p<0.05$) in bothgroup B and C. The differences of IFN-γ levels betweengroups B/C and group A were significant ($p<0.05$). Forthose newborn infants born to mothers who were positivefor both HBsAg and HBeAg, HBIG intervention formothers during late pregnancy, together with combinedtreatment of HBIG and hepatitis B vaccine for infants, gavebetter blocking result of HBV transmission.

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Introduction

Hepatitis B virus (HBV) infection is a challenging disease, and has a high incidence in regions around the world. China is one of the regions with highest incidences of HBV infection, and the disease prevention is still urgent. It was reported that there were about 93 million people in China who were chronic carriers of HBV in 2006, and ~30,000 people became new patients with chronic hepatitis B. Each year the mortality from cirrhosis, liver cancer and other diseases associated with hepatitis B was as many as 300,000 (1,2). As early as 1992, China started to include hepatitis B vaccination in the national planned immunization program, requesting all newborn infants be inoculated with the hepatitis B vaccine (3). Although remarkable achievements have been made, a study found that the rate of non- and hypo-response to hepatitis B vaccine was higher for newborn infants born to mothers who were positive for HBV surface antigen (HBsAg) than that for newborn infants born to normal mothers of general population. These infants had a higher risk to be HBV carriers (4), which caused great physiological and psychological stress to infants themselves and their parents as well. Hepatitis B immune globulin (HBIG) belongs to the category of passive antibodies. HBIG can produce anti-HBs antibodies within a few hours after injection, which neutralize and remove toxins from the body. HBIG provides passive immunization for patients who are already exposed to HBV (5). Studies have shown that combination of the above two immunization approaches had a certain advantage in blocking the mother to infant transmission of HBV (6).

We further studied the effect of HBIG combined with hepatitis B vaccine on blocking HBV transmission between mother and infant, with the aim to confirm and promote this combined immunization remedy from the aspect of immune function, by observing the effect of HBIG on immune cells.

Subjects and methods

Subjects. Ninety newborn infants (single birth) born to maternity patients in Linyi Hospital who were confirmed to be HBsAg-positive in prenatal examination from June 2014 to August 2016 were recruited as research subjects. All maternity patients who met the selection criteria in this study or their

Table I. General data of subjects in the three groups.

Group	HBsAb positive cases	HBsAg/HBeAg positive cases	Maternal age (years)	Mode of delivery		Pregnancy cycle (week)	Infant birth weight (kg)	Infant sex	
				Natural	Caesarean			Male	Female
A	30	11	26.23±6.35	19	11	38.23±2.07	3.43±0.76	16	14
B	30	10	27.09±6.87	18	12	38.12±2.11	3.36±0.68	17	13
C	30	12	26.67±6.33	20	10	38.43±2.43	3.49±0.43	16	14
P-value	>0.05	>0.05	>0.05	>0.05		>0.05	>0.05	>0.05	

HBsAb, hepatitis B virus surface antigen; HBeAg, hepatitis B e-antigen.

Table II. Blocking effects on HBV transmission between mother and infant.

Group	Cases	Cases of successful blockade	Cases of effective blockade	Cases of invalid blockade	Successful blocking rate (%)
A	30	24	3	3	80
B	30	27	1	2	90
C	30	29	0	1	97
Total	90	82	4	5	91

HBV, hepatitis B virus.

Table III. Blocking effects on HBV transmission between mother and infant when mothers were postitive for both HBsAg and HBeAg.

Cases	Cases of successful blockade	Cases of effective blockade	Cases of invalid blockade	Successful blocking rate (%)
11	5	3	3	45
10	7	1	2	70
12	11	0	1	92
22	18	1	3	82

HBV, hepatitis B virus; HBsAb, hepatitis B virus surface antigen; HBeAg, hepatitis B e-antigen.

families signed the informed consent. The research protocol was approved by the Hospital Ethics Committee. Based on the principle of voluntary participation, the subjects were divided into three groups. Thirty subjects in group A received the hepatitis B vaccine at 0, 1 and 6 months after birth at a dose of 10 μg each time. Thirty subjects in group B received an intramuscular injection of 100 IU HBIG 2 h after birth before getting the same treatment as group A. Mothers of 30 subjects in group C received a total of three gluteus maxinus injections of 200 IU HBIG each time at 28 weeks of gestation, 4 weeks and 8 weeks later. The subjects in group C got the same treatment as group B. In Table I are given the general medical data of the maternity patients and the newborn infants in the three groups, including maternal average age, pregnancy cycle, HBsAb-positive cases, HBsAg/HBeAg double positive cases, mode of delivery, average infant birth weight, and infant sex. The data were comparable between groups, and the differences were not statistically significant (p>0.05).

Methods. Maternity patients in group A and B underwent no pretreatment for HBV infection before delivery, but were provided with normal supply of nutrients and adequate psychological counseling. Maternity patients in group C received a total of three gluteus maxinus injections of 200 IU HBIG each time at 28 weeks of gestation, 4 weeks and 8 weeks later. Infants in groups A-C were all vaccinated with hepatitis B vaccine on an immunization schedule of 0, 1, and 6 months with a dose of 10 μg. In addition to receiving the required hepatitis B vaccination, infants in group B and C were required to receive an intramuscular injection of 100 IU HBIG within 2 h after birth. All newborn infants were followed-up until the age of 12 months. Venous blood samples were collected before treatment and at the age of 12 months.

The blood samples were tested for HBV infection (two-and-half pair test) including hepatitis B surface antigen and antibody (HbsAg and HBsAb), hepatitis B e-antigen and e-antibody (HBeAg and HBeAb), and hepatitis B core antibody (HBcAb). Blocking effects of different treatments on HBV transmission were analyzed from these data. Changes of immune cells or related cytokines before and after interventions were also analyzed.

Observed indicators. The blocking effect on HBV mother-to-infant transmission was assessed using the following criteria (7). Among the five above-mentioned tested items, a positive HBsAb only indicated a successful blockade; positive HBsAb, HBeAb and HBcAb together indicated an effective blockade; either positive HBsAg or positive HBeAg indicated an invalid blockade.

Cellular immune functions were evaluated based on following indicators (8). Levels of $CD3^+$, $CD4^+$ and $CD8^+$, and the ratio of $CD4^+/CD8^+$ in serum were measured by streptavidin alkaline phosphatase (SAP) method. Levels of IFN-γ and IL-2 were measured by ELISA before and after immunization.

Statistical analysis. The SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) package was used for statistical analysis.

Table IV. Changes of T lymphocyte subsets in the three groups.

	Group A (%)		Group B (%)		Group C (%)	
Item	24 h after birth	12 months	24 h after birth	12 months	24 h after birth	12 months
$CD3^+$	62.31±9.61	63.96±10.01	60.46±10.65	67.48±9.87	58.73±5.76	62.87±6.82
$CD4^+$	43.52±11.34	45.63±12.45	42.89±10.67	46.03±11.09	40.12±6.18	47.67±5.91
$CD8^+$	16.37±5.91	17.02±4.89	14.65±3.12	15.57±5.12	14.16±4.92	15.36±6.64
$CD4^+/CD8^+$	2.65	2.68	2.86	2.95	2.83	3.1

Measurement data are expressed as mean ± SD, and t-test was used. Enumeration data are expressed as %, and χ^2 test was used. P<0.05 was considered to indicate a statistically significant difference.

Results

Blocking effects on HBV transmission between mother and infant. In Table II are listed the results of blocking effects on HBV transmission between mother and infant, which showed that the three different treatments were all effective. The numbers of infants who were HbsAb-positive were 24, 27 and 29, respectively, in groups A-C, corresponding to blocking success rates of 80, 90 and 97%, respectively. The differences of blocking success rates between group A and B, and between group A and C as well, were statistically significantly (p<0.05), but there was no significant difference between group B and C (p>0.05), though the overall blocking effect in group C was better than in group B.

Blocking effects on HBV transmission between mother and infant when mothers were postitive for both HBsAg and HBeAg. The blocking results are shown in Table III when mothers were postitive for both HBsAg and HBeAg. The successful blocking rate in group A was lower than those in both group B and C, and the differences were statistically significant (p<0.05). In addition, the successful blocking rate in group B was lower than that in group C (p<0.05).

Effects of three blocking approaches on immune cells. The effects of different immunological approaches on immune cells were analyzed by comparing the changes of T lymphocyte subsets from the first immunological intervention to the end of the 12 months follow-up. The results are given in Table IV, showing levels of T lymphocyte subsets $CD3^+$, $CD4^+$ and $CD8^+$ in peripheral blood of the newborn infants in group C were lower than those in group A and B at 24 h after birth, and the differences were significant (p<0.05). The ratios of $CD4^+/CD8^+$ in both group B and C were lower than that in group A (p<0.05).

At the end of the 12 months follow-up, the level of T lymphocyte subset $CD3^+$ in group B was significantly higher than those in group A and C (p<0.05). The $CD4^+$ level and the $CD4^+/CD8^+$ ratio in group C were all higher than those in group A and B (p<0.05), but the differences of these two items between group A and B, and between group B and C, were not significant (p>0.05). The $CD8^+$ level in group C was lower than those in group A and B. The difference with group A was significant (p<0.05), but the difference with group B was not significant (p>0.05).

For all the three immunological approaches, levels of T lymphocyte $CD3^+$, $CD4^+$ and $CD8^+$, and $CD4^+/CD8^+$ ratio were all elevated at the end of follow-up, compared with those at 24 h after birth. The differences of $CD4^+$ levels before and after treatment were significant in group B and C (p<0.05).

Effects of three blocking approaches on IFN-γ and IL-2. Changes of levels of IFN-γ and IL-2 at 24 h and 12 months after birth with corresponding immunological intervention are shown in Figs. 1 and 2. The differences of IFN-γ and IL-2 levels across the three groups were not statistically significant at 24 h after birth (p>0.05). At the age of 12 months, however, the differences between groups B/C who were treated with HBIG combined with hepatitis B vaccine and group A were significant (p<0.05), but the difference between group B and C was not statistically significant (p>0.05).

Discussion

Hepatitis B is a highly contagious disease caused by HBV. The disease can cause damage to a variety of organs at its advanced stage (9). Hepatitis B is widely distributed and hard to cure, thus is one of the major diseases that threaten human life. Its prevention and control measures should be implemented without delay. In the early 21st century, China began to include hepatitis B vaccine in the prevention plan for newborns. A total of three injections of 5 g/dose of recombinant yeast hepatitis B vaccine or 10 g/dose of CHO hepatitis B vaccine were required for neonatal vaccination (10). However, because mother-to-infant transmission ranks first in all HBV transmission routes, there are still many chronic HBV infection cases due to perinatal mother-to-infant transmission (11).

HBIG is a highly effective immunoglobulin collected from plasma of healthy people who are inoculated with hepatitis B vaccine. Treatment of HBIG can reduce the virus level in the blood system, so that less normal cells will be infected due to decreased virus replication, thus enhancing humoral immune function (12). Studies showed that HBV mother-to-infant transmission can be blocked by ~90% if a newborn infant was injected with HBIG after birth and at the same time inoculated with hepatitis B vaccine according to the vaccination schedule (13). In another study, it was reported that HBV infection of newborn infants can be effectively

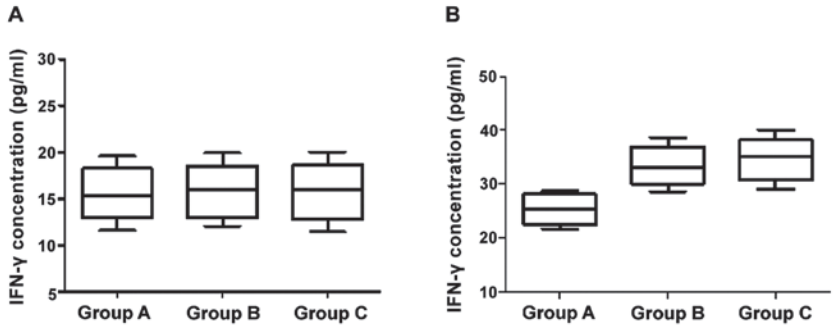

Figure 1. Levels of IFN-γ in the three groups. (A) The levels at 24 h after birth (differences not statistically significant across the three groups, p>0.05); (B) levels at 12 months (differences statistically significant between group B/C and group A, p<0.05).

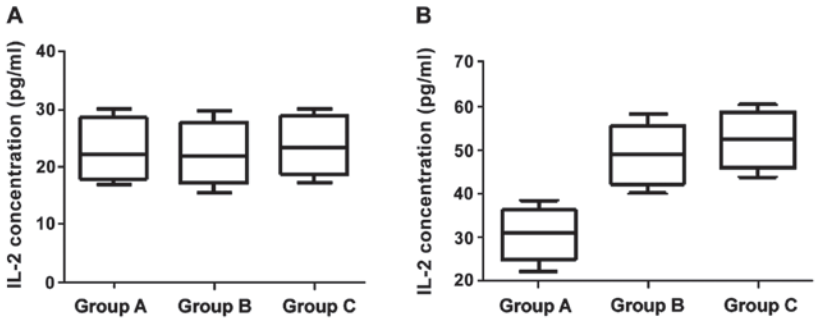

Figure 2. Levels of IL-2 in the three groups. (A) The levels at 24 h after birth (differences not statistically significant across the three groups, p>0.05); (B) levels at 12 months (differences statistically significant between group B/C and group A, p<0.05).

prevented and blocked if maternity patients were treated with HBIG before delivery and additionally newborn infants were treated with HBIG combined with hepatitis B vaccination (14). In the present study, the effect of treatment approaches for both mothers and infants on blocking HBV transmission was explored, in which mothers were treated with HBIG before delivery and infants were treated with HBIG combined with hepatitis B vaccination. The theoretical base of this study is stated below (15,16). Twenty weeks after gestation, the placenta possesses the function of transferring maternal IgG antibodies to the fetus by active transport. This is even more obvious in the late stage of pregnancy. In addition, various fetal organs generally reach their complete development at this stage, allowing drug administration to be safer to the fetus. Thus prenatal intervention measures at this time can be effective in blocking the transmission of HBV in the uterus. The results of this study are consistent with this theory.

The origin and progression of hepatitis B is directly related to the immune response, which is mediated and regulated by immune cells and their secreted cytokines. Therefore, the study of the impact of immune intervention measures on immune cells is important to further understand the disease. It was reported that the main immunological indicators of physical immunity were T lymphocyte levels and the ratios of T cell subsets (17). T lymphocytes are important immunologically active cells, whose subsets, the $CD4^+$ cells and $CD8^+$ cells, can modulate the activity of other immune cells (18). Lower than normal T lymphocyte level and its subset ratios indicate an HBV infection and immune modulation disorder. Measures of immunization intervention are implemented in order to improve physical immune function. IFN-γ is a cytokine that is secreted by helper T cells, specifically by Th1 cells. It has been shown to be a crucial player in the immune response (19). Immunization intervention increased IFN-γ levels in serum, which inhibited replication of HBV and improved immune function of the body (20).

In this study, the effects of three different immunization approaches on blocking HBV mother-to-infant transmission were compared. The results showed that the blocking effect of HBIG combined with hepatitis B vaccination was better than the blocking effect of hepatitis B vaccination alone for newborn infants born to mothers who were HBsAg-positive. For those newborn infants born to mothers who were positive for both HBsAg and HBeAg, HBIG intervention for mothers during late pregnancy, together with combined treatment of HBIG and hepatitis B vaccination for infants, gave better blocking result of HBV transmission, than the same treatment for infants only and not affecting the mothers. In addition, in the study of effects of different immunization approaches on immune cells, it was found that the treatment approaches for both mother and infant increased the levels of $CD4^+$ and other T cell subsets, the subset ratios, and the IFN-γ cytokine in infants. These changes made a great impact on immune cells and thus improved the immune function.

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