Interruption of HBV intrauterine transmission: A clinical study

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MATERIALS AND METHODS

Subjects
One hundred and fifty one pairs of women and their newborns who followed the antenatal care were selected and admitted for labor in our hospital from January of 1999 to December of 2001. These pregnant women were HBsAg positive, with normal liver and kidney function. Serial tests were negative for HAV, HCV, HDV and HEV in these women and no other severe complications were found and no other drugs, including the ones that were studied, anti-virus, cytotoxic, steroid hormones, or immune regulating drugs were administrated. The patients were randomly allocated into 3 groups. There were 56 patients in the HBIG group (22 were both HBsAg and HBeAg positive) and 43 in the lamivudine group (33 were both HBsAg and HBeAg positive). There were 52 patients in the control group (17 were both HBsAg and HBeAg positive). No significant differences were found in age, race, time of gestation and parturition, gestational age, way of delivery, and incidence of threatened abortion, threatened labor or pregnancy-induced hypertension syndrome (PIH). The 151 pregnant women delivered 151 newborns.

Methods
Patients in the HBIG group were administered HBIG 200 IU intramuscularly (im.) from 28-wk of gestation, once every 4 weeks till labor. Patients in the lamivudine group were administered 100 mg (po.) lamivudine orally daily till the 30th day after labor. Patients in the control group were given no specific treatment. Blood specimens were tested for HBsAg, HBeAg, and HBV-DNA in all maternities at 28-wk of gestation, before delivery, and in their newborns 24 hours before the administration of immune prophylaxis.

RESULTS

HBsAg and HBeAg were assessed by ELISA, the assay kits were produced by Zongshanz Biological and Engineering Co. Ltd. HBV-DNA was assessed by fluorogenic quantitative polymerase chain reaction (FQ-PCR), and the assay kits were produced by Da’an Gene Diagnosis Center, Sun Yat-Sen University.

Before the administration of positive and/or active prophylaxis at 24 hours after delivery, intrauterine HBV infection would be considered if HBsAg and/or HBeAg were tested positive in neonatal peripheral blood.

Statistics
The t-test and χ2 test were used to analyze our data using Excel software. Statistical significance was set at P<0.05. HBV DNA values were expressed as ±s, and neonatal intrauterine HBV infection rates were expressed as percentage of total cases in each group.

RESULTS

Changes of HBsAg, HBeAg and HBV DNA
HBsAg turned negative in 1 case of the HBIG group, but HBeAg turned negative in no case. HBsAg and HBeAg turned negative in 1 case of the lamivudine group. No cases turned negative of HBsAg or HBeAg in the control group.

Before administration of agents, there was no significant difference in the values of HBV DNA among 3 groups.
administration and follow-ups. There were no significant differences among the 3 groups in the highly risk factors (threatened abortion, threatened premature labor) or age, time of gestation and delivery, pregnant complication, medical or surgical complication, gestational age at labor or way of delivery.

It has been clinically accepted to administer joint immune reagents (HBIG together with HBV vaccine) to neonates with high risks, but the immune failure rate is still about 10-20%[15,16], the main reason is intrauterine infection. So, it is important to study the mechanism of HBV intrauterine infection, and we further investigated the intrauterine prevention and interruption of HBV infection.

**HBIG** is a highly effective immune globulin[17], which is purified from highly effective plasma or serum taken from healthy individuals after the use of HBV vaccine. HBs antibody can bind HBsAg, activate the complimentary system at the same time and strengthen humoral immune, clear HBV, and reduce the number of virus in the maternal blood. It can prevent and decrease the incidence of normal cell infection and might reduce HBV copy in the body. Placenta has the function of transmitting antibody in the form of IgG to the fetus. It is suggested that after maternal administration of HBIG (im.), HBsAb can be transmitted to fetus, which makes it possible for the fetus to obtain the protection of intrauterine passive immunization and to prevent intrauterine infection[18]. The results of this study suggest that regular administration of HBIG (im.) to HBV positive pregnant women might reduce the amount of HBV DNA in blood, and neonatal intrauterine infection rate also reduced significantly when compared with control group. DNA polymerase of HBV has many functions in the process of viral replication. After infection of liver cells by HBV, the incomplete double strand DNA integrates into a complete one, enters the nuclei, forming super helix covalent closed circular DNA (cccDNA). cccDNA is extremely stable, and is the resource of viral DNA and directs the formation of viral protein. The whole mRNA replicated from cccDNA model can form a single strand minus-DNA by reverse-transcription of the HBV DNA polymerase. This DNA can form incomplete double strand DNA through DNA polymerase. The latter can also integrate with antigen proteins in the endoplasm, forming new, contagious mature viral particles and be released into blood, or migrate into the nuclei to supply cccDNA there. The multiple functions of HBV polymerase enable it to become one of the most prosperus anti-virus targets.

Lamivudine is a potent anti-virus nucleotide analogue to HBV and HIV. Through competitive inhibition of HBV DNA polymerase and formation of new HBV DNA strand, it can terminate the synthesis of new strand[19,20]. After several days of the administration of lamivudine, the level of HBV DNA drops dramatically, and throughout the treatment, HBV DNA will be suppressed continuously. It can reduce the necrosis and inflammation of the liver and bring ALT level to normal without significant side effects or malformation-causing effects[21-31]. We found the amount of HBV DNA in blood and the rate of neonatal intrauterine infection after administration of lamivudine in the 3rd trimester were significantly lower than that in control group. This suggests that administration of lamivudine of HBV positive pregnant women in the 3rd trimester can effectively decrease the rate of intrauterine HBV infection.

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**Table 1** Comparison of HBV DNA values before and after administration of the reagents

| Group     | Log10 HBV DNA before administration of drugs (copies/ml) | Log10 HBV DNA before labor (copies/ml) | Minus value of log10 HBV DNA before and after administration of agents (copies/ml) |
|-----------|--------------------------------------------------------|----------------------------------------|----------------------------------------------------------------------------------|
| HBIG      | 7.38±1.17<sup>a</sup>                                  | 5.28±2.77<sup>bd</sup>                 | 2.09±2.28<sup>d</sup>                                                            |
| Lamivudine| 7.49±0.54<sup>a</sup>                                  | 5.33±1.34<sup>bd</sup>                 | 2.16±1.27<sup>d</sup>                                                            |
| Control   | 7.05±1.29<sup>a</sup>                                  | 6.23±3.66<sup>bd</sup>                 | 0.82±2.73<sup>d</sup>                                                            |

<sup>a</sup>P<0.05 vs other groups; <sup>b</sup>P<0.05 between HBIG group and lamivudine group; <sup>d</sup>P<0.05 vs HBIG group or lamivudine group;<sup>bd</sup>P<0.05 (before vs after administration).

**Incidence of HBV intrauterine infection**

Three newborns were HBsAg positive, and 7 cases were HBsAg positive, one of them was doubly positive for HBsAg and HBeAg in HBIG group. Corresponding cases in lamivudine group and control group were 1, 7, and 1, or 8, 11, and 2 respectively. The infection rates of HBIG, lamivudine, and control groups were 16.1 %, 16.3 %, and 32.7 %, respectively. There were significant differences between the incidence of HBV intrauterine infection in either reagent administrated group and control group (P<0.05), while there was no significant difference between HBIG group and lamivudine group (P=0.05). (Table 2).

**Table 2** Incidence of neonatal intrauterine infection in 3 groups

| Group     | n  | HbsAg(+)n | HBeAg(+)n | Intrauterine infection |
|-----------|----|-----------|-----------|------------------------|
| HBIG      | 56 | 3         | 7         | 9                      | 16.1<sup>c</sup> |
| Lamivudine| 43 | 1         | 7         | 7                      | 16.3<sup>c</sup> |
| Control   | 52 | 8         | 11        | 17                     | 32.7<sup>c</sup> |

<sup>c</sup>P<0.05 between HBIG group and lamivudine group; <sup>d</sup>P<0.05 vs HBIG group or lamivudine group.

**Safety**

There were no incidences of fever, rigor, rash, or other complaints and dysfunction of the liver and kidney in subjects throughout administration and follow-ups. There were no significant differences in gestational age, severity of postpartum hemorrhage, rate of cesarean section, neonatal weight, neonatal height, circumference of neonatal head and Apgar score (P>0.05).

**DISCUSSION**

There are several thoughts about the mechanisms of HBV intrauterine transmission, including placental infection[8], placental exudation and transudation[9,11], peripheral blood monocyte (PBMC)[12], infection, fratern transmission, etc. Infection through placenta is the most active pathway in maternity-fetus transmission. It is suggested that infection mainly occurs in the 3<sup>rd</sup> trimester. This might be resulted from the fact that the layer of trophoblastic cells becomes thinner and turns into chorion-vessel membrane, which makes it easier for HBV to pass the placental barrier[13]. The organs of fetus during this period have already developed, therefore, it is safe for the administration of reagents. So we chose this period to begin the interruption of infection. Lamivudine (po.) or HBIG (im.) was administered from 28-wk of gestation.

Barrier-destroying factors, such as threatened abortion, threatened premature labor and TORCH (toxoplasmosis, others, rubella, cytomegalovirus, herpes) infection, were the highly risk factors for HBV intrauterine infection[14]. It is generally considered that intrauterine infection might be the general effect of maternity and virus. In this study, there were no significant differences among the 3 groups in the highly risk factors (threatened abortion, threatened premature labor) or age, time of gestation and delivery, pregnant complication, medical or surgical complication, gestational age at labor or way of delivery.

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**Note**

(P<0.05). But there was significant difference between the values of HBV DNA in HBIG group and lamivudine group after administration of either reagent respectively (both values reduced, P<0.05). The reduction of value before and after administration of the reagents was significantly different among the administered groups and control group (P<0.05). (Table 1).
As a passive antibody, the main effect of HBIG is to neutralize HBV in the body, prevent and decrease infection of normal cells\(^{[1]}\), while lamivudine is a potent anti-virus agent, which can suppress the replication of HBV actively, decrease HBV level during pregnancy. Our data showed that the neonatal infection rates, after these two reagents were used in the 3rd trimester to interrupt intrauterine HBV infection, were 16.1 % and 16.3 %, respectively, with no significant difference between these 2 groups (\(P>0.05\)). But compared with control group, the infection rates of both groups were significantly lower. These data indicate that both of them are safe and effective in the interruption of intrauterine HBV infection.

We found in our previous studies that HBV DNA level in maternal serum was an important factor for intrauterine infection\(^{[2]}\), and maternal serum was an important factor for intrauterine infection rates, after these two reagents were used in the 3 trimesters\(^{[3]}\), which can suppress the replication of HBV actively, decrease viral load\(^{[4]}\). These data indicate that both of them are safe and effective in the interruption of intrauterine HBV infection. Further studies are required to improve our understanding about this problem.

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