Genetics and Molecular Research

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Efficacy and safety of nucleoside analogues in preventing vertical transmission of the hepatitis B virus from father to infant

L.-H. Cao[1], P.-L. Zhao[1], Z.-M. Liu[1], S.-C. Sun[2], D.-B. Xu[1], J.-D. Zhang[1] and Z.-H. Shao[1]

[1]Third Hospital Liver Disease Center of Qinhuangdao, Qinhuangdao, Hebei, China
[2]Women and Children's Hospital of Qinhuangdao, Qinhuangdao, Hebei, China

Corresponding author: L.-H. Cao
E-mail: caolihua_clh@163.com

Genet. Mol. Res. 14 (4): 15539-15546 (2015)
Received August 8, 2015
Accepted October 2, 2015
Published December 1, 2015
DOI: http://dx.doi.org/10.4238/2015.December.1.4

ABSTRACT. We examined the efficacy and safety of nucleoside analogues in preventing the vertical transmission of hepatitis B virus (HBV) from father to infant. We included 201 patients who visited the liver clinic of our hospital. The patients were positive for HBV surface antigen (HBsAg), HBeAg, anti-HBc, and HBV DNA; 189 patients (94%) had abnormal liver function. In all couples, the fathers were HBV DNA-negative and had normal liver function, and the mothers were anti-HB-positive before pregnancy. The control group comprised 188 couples who visited our hospital during the same time period. The fathers in the control group were positive for HBsAg, HBeAg, anti-HBc, and HBV DNA. The mothers were HBsAg-negative and anti-HBs-positive. No infants in the case group were HBsAg-positive and HBV DNA-positive, and all were anti-HBs-positive, indicating that father to infant HBV vertical transmission was prevented in the case group. In the control group, 147 of 188 newborns (78.2%) were anti-HBs-positive at birth, 28 (14.9%) were HBV DNA-positive, and 19 (10.1%)

were HBsAg-positive. A significant difference was observed between the two groups. No statistically significant difference was observed in the gestational age, birth weight, birth length, 1-min and 8-min Apgar score, jaundice, other internal and surgical diseases, delivery mode, and other birth information between the neonates born to couples in the case and control groups; there were no fetal malformations and stillbirths in the two groups. Our results showed that administration of antiretroviral therapy to HBV DNA-positive fathers before pregnancy can cause a decrease in the viral load and prevent father to infant HBV vertical transmission. The use of antiviral nucleoside analogues before pregnancy was safe in fathers, and the fathers who wanted children could continue to use anti-viral therapy. The sample size in our study was small, and further studies with a large sample size and longer follow-up time are required for determining the use of nucleoside analogues from the point view of prenatal and postnatal care.

Key words: Nucleoside analogues; Hepatitis B virus; Antiviral therapy; Father to infant vertical transmission

INTRODUCTION

The incidence of hepatitis B virus (HBV) infection is high in China. The nationwide program of vaccination against hepatitis B has gradually decreased the incidence of HBV transmission. A national hepatitis epidemiological survey in 2006 showed that 7.18% of the general population in the age group of 1-59 years are carriers for the hepatitis B surface antigen (HBsAg), and nearly 0.96% of children under 5 years old are carriers for HBsAg (Liang et al., 2009a,b). An investigation by Blumberg (1977) using the pedigree method showed that HBV DNA may be integrated into the host germ cell genes, and then it can pass on to the offspring. Several previous studies have shown the presence of HBV DNA in the semen and sperm. The development of transgenic technology has enabled detailed examination of the sperm as a pathway for transmission of HBV (Araki et al., 1989; Huang et al., 2002). The egg of the golden hamster is fertilized using the sperm of patients infected with HBV; in human sperm chromosome specimens, a virus probe hybridization signal can be observed. During fertilization, the human sperm carrying the HBV DNA is not selectively eliminated, and it can successfully complete the fertilization process. The sperm carrying HBV DNA can actively suck in the virus; it is generally considered that the motility of HBV-infected sperm is not affected, and it can successfully combine with the egg for further development (Ali et al., 2005). Ali et al. (2006) and others have confirmed that the sperm-mediated HBV gene can be expressed in the early embryonic cells, and these results provided direct evidence that HBV can be transmitted to the offspring from the parents. Father to child HBV transmission pathways can be divided into horizontal and vertical transmission pathways. In the case of horizontal transmission, because the neonatal immune system is weak, a father carrying HBV can pass the virus through daily contact to the newborn. Horizontal transmission of HBV can be prevented by concomitant injections of hepatitis B immunoglobulin (HBIG) and hepatitis B vaccine (HBVac). However, vertical transmission is transmitted by germ cells. In particular, a father that is “big three-positive” is more likely to transmit HBV to the infant via vertical

transmission; father to child transmission is the second most important pathway of HBV vertical transmission (Komatsu et al., 2009). Prevention of father to infant vertical transmission of HBV continues to be challenging and further studies are required to address this issue.

Immunohistochemical methods are one of the main strategies of blocking HBV vertical transmission in current clinical treatment, but immune failure continues to be observed in 20% of neonates because of the presence of HBV DNA in the serum of pregnant women. When the maternal serum HBV DNA level is greater than 108 IU/mL, the rate of intrauterine infection is as high as 43% even with immunological methods. A decrease in the serum HBV levels below 10^6 IU/mL is associated with a 30% reduction in the risk of mother to child HBV transmission (Lin et al., 2000). Efficient and safe anti-HBV drugs should be used in pregnant women with a high viral load to inhibit HBV replication. Previous studies on preventing mother to infant HBV transmission have shown that lamivudine plays a role in preventing HBV intrauterine infection (van Zonneveld et al., 2003). Studies on preventing mother to infant HBV transmission showed that nucleoside antiviral drugs can also be used to block father to infant HBV transmission. The aim of this study was to evaluate the clinical efficacy and safety of HBV nucleoside analogues to inhibit father to infant HBV transmission.

MATERIAL AND METHODS

Case group and control group selection

In the case group, outpatients in our hospital were consecutively selected from March 2006; we selected 201 couples willing to prepare for childbirth who had good compliance and were willing to regularly visit our hospital and receive follow-up examinations. We examined the HBV markers (HBVM) of the fathers during the visits. The fathers were HBsAg-positive, HBeAg-positive, anti-HBc-positive, and HBV DNA-positive; 189 patients had abnormal liver function, which accounted for 94.0% (189/201) of the patients. Fathers who were HBsAg-positive, anti-HBe-positive, anti-HBc-positive or HBsAg-positive, anti-HBc-positive, and HBV DNA-positive were not included in this group. The mothers had normal liver functions, and they were anti-HBs-positive or anti-HBs-positive, anti-HBc or anti-HBs-positive, anti-HBe-positive, and anti-HBc-positive; we included 139 patients (previous vaccination with HBVac or acquired immunity via previous HBV infection), and they accounted for 69.2% (139/201) of the patients. Sixty-two patients (30.8%) were anti-HBs-negative. The inclusion criteria for all couples in the case group were an HBV DNA-negative father with normal liver function and anti-HBs-positive mothers.

We selected 188 couples in the control group from 1957 couples who received antenatal examination in Qinhuangdao Maternal and Child Hospital from March 2006. The liver functions of fathers were all normal; they were HBsAg-positive, HBeAg-positive, anti-HBc-positive, and HBV DNA-positive; 13 patients had a viral load less than or equal to 10^6 IU/mL; 175 patients had a viral load greater than 10^6 IU/mL. The levels of HBVMs were not detected in the mothers before pregnancy, and the mothers had no memory of HBVac injection. The liver function of patients in the control group was normal. The patients were HBsAg-negative, anti-HBs-positive or anti-HBs-positive, anti-HBc-positive or anti-HBs-positive, anti-HBe-positive, anti-HBc. While HBVM were negative, anti-HBs-negative, only anti-HBc-positive were not included in this group of the study.

All selected couples met the following conditions: serum A, C, D, and E hepatitis

viruses and human immunodeficiency virus tests were negative; no alcoholic liver disease and autoimmune liver disease; normal renal function; and agreed to sign the written informed consent forms. Fathers had a history of 2-23 years. Venous blood samples were collected from the newborns at birth. The umbilical cord blood was considered as residual blood in the placenta and umbilical cord, and the fetal umbilical cord was ligated to prevent mixing with the mother's blood. The other factors that could potentially contaminate the umbilical cord blood were placenta previa, placental abruption, and various other factors associated with Cesarean delivery.

Observed indicators

Case group

The liver function, HBVMs, and HBV DNA of the fathers were detected before and 1, 3, 6, 9, and 12 months after antiretroviral treatment. The liver function and HBVMs were detected in the mothers before pregnancy. Sixty-two patients were anti-HBs-negative. Anti-HBs were detected before HBVac administration and 1 month and 7 months after HBVac administration.

Control group

Liver function and HBVMs were detected in the mothers, and liver function, HBVMs, and HBV DNA were detected in the fathers.

Venous blood of infants was immediately drawn after birth to detect HBVMs and HBV DNA.

Detection methods

Detection of HBVMs and HBV DNA in newborns was performed using the same methods that used in the case group and control group. Electrochemiluminescence method (Cobas-e-411 automatic electrochemiluminescence immunoassay analyzers; Roche, USA) was used to detect HBVMs. HBV DNA was analyzed using real-time quantitative polymerase chain reaction (PCR) by using the reagents provided by the Sheng Xiang Biotechnology Co., Ltd., and the quantitative PCR instrument (SLAN) from Hunan Sheng Xiang Biotechnology Co., Ltd. was used for detection.

Treatment

In the 201 couples in the case group, HBVM detection was performed in the mothers. Sixty-two mothers were anti-HBs-negative, and pre-pregnancy vaccination of HBVac was performed (0, 1, and 6 months; 20 or 40 µg recombinant yeast) to ensure anti-HBs before pregnancy. HBVac injection was not administered to 139 anti-HBs-positive mothers; 201 mothers were anti-HBs-positive before pregnancy. Treatment with the antiviral nucleoside analogue was administered to 201 fathers carrying HBV DNA; 52 patients received lamivudine (100 mg/day), 24 patients received adefovir (10 mg/day), 87 patients received telbivudine (600 mg/day), and 38 patients received entecavir (0.5 mg/day).

All mothers among the 188 couples of the control group were anti-HBs-positive, and no tests were performed during the pregnancy; 188 fathers were HBV carriers, and they received no treatment before pregnancy.

Statistical analysis

The SPSS16.0 statistical software was used for statistical analysis. Continuous data were compared using the Student *t*-test, and categorical data were compared using the chi-square test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Basic characteristics of the couples in the two groups are shown in Table 1 and Table 2, and complications during pregnancy are shown in Table 3. Of the 201 patients, 167 became pregnant during antiretroviral treatment; 34 fathers had the pregnancy 3-6 months after they were HBV DNA-negative and after withdrawal of the antiviral drug (Table 4). Of the 167 patients in the case group (pregnancy during medication), 121 were HBV DNA-negative before pregnancy. None of the newborns were HBsAg-positive, and none of them were HBV DNA-positive. Normal liver function was observed in 46 patients but no HBV DNA-negative turning, the values were within 1.1 x 10^3 to 8.9 x 10^4 IU/mL. No newborn was HBsAg-positive born to couples who were HBV DNA-positive while pregnancy was not [illegible]. No newborn was HBV DNA-positive. In the control group, 147 of 188 newborns were anti-HBs positive at birth, which accounted for 78.2% (147/188) of the couples. Twenty-eight newborns (14.9%) were HBV DNA-positive, and 19 (10.1%) were HBsAg positive. Thirteen newborns (6.91%) were anti-HBs and HBV DNA-negative (Table 5).

Table 1. Basic characteristics of fathers in the two groups.

	Case group	Control group	χ^2 or *t*	P value
N	[illegible]	188	*t* = 0.612	0.574
Age (years)	29 ± 2.1	28 ± 1.9	*t* = 0.427	0.691
Height (cm)	176 ± 5.1	174 ± 6.3	*t* = 0.231	0.829
Weight (kg)	65 ± 5.1	66 ± 5.5	*t* = 0.231	
Antiviral treatment before pregnancy	201	0	χ^2 = 389	0.000

Table 2. Basic characteristics of mothers in the two groups.

	Case group	Control group	*t*	P value
N	201	188	0.85	0.443
Age (years)	25 ± 2.3	26 ± 2.0	0.453	0.674
Height (cm)	160 ± 5.1	158 ± 5.7	0.444	0.680
Weight (kg)	55 ± 5.1	53 ± 5.9	0.00	1.00
Pregnancy time	2 ± 1.0	2 ± 1.0		
Quantitative anti-HBs before pregnancy	497 ± 35.7	505 ± 38.8	0.263	0.806

Table 3. Common complications during pregnancy in the two groups.

	Case group	Control group	χ^2	P value
N	201	188		
Hypertension	6	5	0.037	0.874
Pre-eclampsia	0	0		
Diabetes	0	0		

Table 4. General characteristics of neonates at birth.

	Case group (N = 201)	Control group (N = 188)	χ^2 or t	P value
Pregnancy week	39.21 ± 1.21	39.01 ± 1.32	t = 0.193	0.856
Weight at birth (kg)	3.31 ± 0.31	3.32 ± 0.32	t = 0.039	0.971
Height at birth (cm)	49.71 ± 1.57	49.62 ± 1.51	t = 0.072	0.946
Gender (M/F)	98/69	95/66	χ^2 = 0.004	0.953
1-min Apgar score	9.85 ± 0.51	9.79 ± 0.49	t = 0.147	0.890
8-min Apgar score	9.86 ± 0.57	9.83 ± 0.58	t = 0.064	0.952
Jaundice	17	16	χ^2 = 0.000	0.985
Other internal and surgical diseases	0	0		
Delivery mode (Sectional/Cis)	104/97	98/90	χ^2 = 0.006	0.939

Table 5. Levels of hepatitis B virus biomarkers (HBVMs) in the newborns at birth in the two groups.

	Case group	Control group	χ^2	P value
N	201	188		
Anti-HBs (+)	201	147	138.278	0.000
HBsAg (+)	0	19	21.357	0.000
HBV DNA	0	28	32.2[illegible]	0.000

DISCUSSION

In 1985, Hadchouel et al. used molecular hybridization to determine that integrated HBV DNA was present in the sperm of three patients with acute hepatitis B infection, which demonstrated the phenomenon of father to infant vertical transmission. In addition, Tajiri et al. (2007) found evidence of father to child hepatitis transmission at the molecular level. The possibility of father to child transmission of HBV has increased in the recent years. Huang et al. (2003) showed that HBV affects reproductive cadre sperm chromosome mutation. The integration between HBV DNA and sperm chromosomes was observed at multiple loci and was random, and the majority of the integration was at multiple loci. This further increased the instability of chromosome, which suggested that HBV infection will have a widespread impact by altering the genetic composition across generations. Englert et al. (2004) reported that with assisted reproductive technology (ART), washed gradient centrifugation can be used to reduce the HBV viral load and avoid contamination of embryos, which could help to reduce the spread of hepatitis B. However, ART showed some difficulties in clinical implementation to effectively prevent father to infant vertical transmission. Thus, a convenient and effective method to block father to infant vertical transmission is required.

Status of newborns at birth

Nucleoside analogues can act on the polymerase region of HBV, and can substitute similar structured nuclei extending toward the desired nucleoside polymerase chain in the viral replication process. Subsequently, extension of the chain is terminated to inhibit viral replication. Thus, this treatment can markedly inhibit viral replication. In recent years, nucleoside (acid) antiviral drugs have been widely used in the treatment of chronic hepatitis. However, the duration of treatment is very long, and only one-fourth of the patients may discontinue the drugs within 5 years. In addition, many patients being treated with these agents will face fertility problems. The interferon antiviral treatment will affect the sperm, and previous studies

have shown that in male patients undergoing long-term interferon and ribavirin therapy, the sperm count was reduced, sperm were abnormal, and the sperm DNA chromosome structure showed some abnormalities. These effects usually returned to normal gradually 8 months after discontinuation of treatment, although during the treatment, the sperm were not suitable for fertility. To date, no study has shown that nucleoside analogues can cause sperm abnormalities at therapeutic doses. We used 4 nucleoside analogs in this study: lamivudine, adefovir, telbivudine, and entecavir. Reproductive studies in animals showed that lamivudine was not teratogenic, and it had no effect on male and female reproductive capacity. Animal studies showed that adefovir had no effect on the fertility of male and female animals. Telbivudine did not show genotoxicity in *in vivo* and *in vitro* studies. Gene mutation tests in mammalian cells did not show a clastogenic effect. Although male and female rats received a systemic dose 14 times, the therapeutic dose of telbivudine in humans, they did not show impaired fertility. Reproductive toxicity studies showed that entecavir did not affect fertility in male and female rats. Toxicological studies showed that when the dose was 35 times or more than the human dose, degeneration of the vas deferens was found in rodents and dogs. Studies in monkeys showed no change in the testis. In summary, the four kinds of drugs were determined to be relatively safe in humans. Toxicity and potential carcinogenicity were found to be an issue only if the drug was overdosed. Our results showed no statistically significant difference in the gestational age, birth weight, birth length, 1-min and 8-min Apgar score, jaundice, other internal and surgical diseases, mode of delivery, and other birth data between the case and control groups. There were no fetal malformations or stillbirths in the two groups. Further, neonatal infant HBV vertical transmission and an HBsAg-positive father showed no significant effects on the indicators above (Table 4). Unlike our study, previous studies showed that the rate of sperm chromosome aberrations in HBV-infected fathers was significantly higher than that in healthy controls, which increases the rates of infertility, miscarriage, stillbirth, perinatal child mortality, and the risk of fetal malformations (Jarvezey et al., 2002; Huang et al., 2003; Vicari et al., 2006; Ye et al., 2014). Our results showed that the use of antiviral nucleoside analogues before pregnancy was safe in fathers. When the benefits outweighed the risks, the fathers who wanted to have children could continue to use anti-viral therapy. However, the sample size in our study was small and more cases and longer follow-up times, as well as further in-depth assessment from the point view of prenatal and postnatal care are required in the future.

HBV father to infant vertical transmission

Beginning at 20 weeks of pregnancy, the placenta has an active immunoglobulin G antibody transmission function from the mother to the fetus. Ayoda and Johnson (1987) found that in 72 patients with no HBV infection, in which pregnant women received HBVacs, 59% of neonates were HBsAb-positive at birth, indicating that fetal immunity was acquired successfully.

Previous studies show that the neonatal population with an HBsAg-positive father have a higher prevalence of HBV infection, and they are at a high risk for HBV infection (Ali et al., 2005; Takegoshi and Zhang, 2006; Komatsu et al., 2009). However, the rate of transmission differs across various studies, and no consistent conclusions can be drawn (Wang et al., 2003; Cai and Zhu, 2013). This may be attributed to the different diagnostic criteria used by the institutes; on the other hand, the subjects selected for the study may themselves be different. Our results showed that none of the newborns were HBsAg-positive, HBV DNA-positive case, and anti-HBs-positive, and father to infant HBV vertical transmission was successfully

prevented. By contrast, in the control group, 147 out of 188 newborns (78.2%) were anti-HBs-positive at birth. Twenty-eight (14.9%) newborns were HBV DNA-positive, and 19 newborns (10.1%) were HBsAg-positive. A significant difference was observed between the two groups (Table 5). Our results suggested that administration of antiretroviral therapy to an HBV DNA-positive father before pregnancy markedly decreased the viral load and successfully prevented father to infant HBV vertical transmission. The sample used in this study was small, and further studies with a larger sample size should be performed in the future. Quantification of HBV DNA in fathers and anti-HBs in mothers is required for further stratified observations.

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