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【 ORIGINAL ARTICLE 】

The Effect of the Hepatitis B Vaccine Derived from Genotype C on Infants Born to Mothers Infected with Genotype D

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Abstract:

Objective There is a paucity of information on whether the hepatitis B virus (HBV) vaccine, derived from HBV genotype C, can prevent mother-to-child transmission of HBV genotype D. The aim of this study was to clarify this issue.

Methods The subjects consisted of 25 children (8.5±4.1 years old, 7 males, 18 females), born to 17 mothers who were chronically infected with HBV genotype D. Of these, 20 children were inoculated with the genotype C-derived vaccine, one was inoculated with the genotype A-derived vaccine, and one was inoculated with both the A- and C-derived vaccines. Information on the type of vaccine given to the remaining three children was not available. The serum levels of HB surface antigen (HBsAg), antibody to HBsAg (anti-HBs), and antibody to HB core (anti-HBc) of the children, as well as HBV markers of the mothers, were examined.

Results All mothers were positive for HBsAg (6,563±11,005 IU/mL), negative for HBeAg, and positive for anti-HBe. HBV-DNA levels (log IU/mL) were <3.3 in 7 mothers, 3.3-4.3 in 9 mothers, and >4.3 in one mother. HBsAg and anti-HBc were negative in all children, regardless of the type of vaccine used. Anti-HBs were positive in 13 children and negative in 12.

Conclusion All children born to mothers infected with genotype D, including 20 who were inoculated with the genotype C-derived vaccine, were negative for both HBsAg and anti-HBc. These results suggest that the genotype C-derived HB vaccine is effective in preventing mother-to-child transmission from mothers infected with HBV genotype D.

Key words: anti-HBc, genotype, hepatitis B virus, mother-to-child transmission, vaccine

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Introduction

Hepatitis B is a major global health problem; it is estimated that 257 million people were chronically infected with the hepatitis B virus (HBV) in 2015, and 887,000 people died as a result (1). HBV is transmitted by percutaneous or mucosal exposure to infectious blood or bodily fluids. Transmission can occur perinatally from mother to child and from person to person. Infants born to mothers infected with HBV are at high risk of acquiring this infection; therefore, the prevention of perinatal HBV transmission is crucial. A 3-dose series of HB vaccination with or without hepatitis B immune globulin (HBIG) is recommended to infants born to mothers positive for hepatitis B surface antigen (HBsAg), and infants whose anti-HBs remains <10 mIU/mL by post-vaccination testing are recommended to receive revaccination (2, 3).

In Japan, the nationwide Mother-to-Child Transmission Prevention Project (MCTP) was launched in 1986. In this

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project, babies born to mothers positive for HBsAg and hepatitis B e antigen (HBeAg) in serum received HBIG at birth, and at 2 months of age, as well as vaccines at 2, 3, and 5 months of age (4-6). In 1995, this project was expanded to include children from HBsAg-positive and HBeAg-negative mothers, while omitting HBIG administration at 2 months.

It has not been fully clarified whether immunization with the HB vaccine derived from single-genotype HBV can prevent infection with different (non-vaccine) HBV genotypes. Many reports have shown that universal vaccination with HBV vaccines (most of which are derived from genotype A) is highly effective; thereby reducing the rate of chronic infection in children in many countries where a variety of HBV genotypes are circulated, thus indicating the efficacy of HB vaccines against different genotypes (1, 2, 7)1. However, several reports have also reported a reduced efficacy of the HB vaccine against different genotypes (8-10).

Thus far, 10 HBV genotypes with different geographical distributions have been reported (11, 12). In Japan, genotypes C and B are the most prevalent domestic genotypes; however, the incidence of genotype A is increasing, genotype D is also found among the Japanese population (13-16). Two types of HB vaccines are available in Japan (9, 17). One is a genotype C-derived vaccine (Bimmugen®, Kaketsuken, Kumamoto, Japan), and the other is derived from genotype A (Heptavax®-II, MSD, Kenilworth, USA). In the present study, we aimed to clarify whether different genotype-derived vaccines, specifically the HB vaccine derived from genotype C, can prevent mother-to-child transmission in children born to mothers infected with genotype D.

Materials and Methods

Study design and ethics

This study was designed as a prospective, observational cohort study to investigate whether genotype C- or genotype A-derived HB vaccination in neonates born to mothers chronically infected with genotype D can prevent mother-to-child transmission. This study aimed to prospectively investigate HBsAg, anti-HBs, anti-HBc, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the type of HB vaccine given to children born to mothers chronically infected with genotype D HBV and who had been inoculated with the HB vaccine according to the protocol of the nationwide "mother-to-child transmission prevention project". The data on the type of HB vaccine (genotype C-derived, or genotype A-derived vaccine), date of vaccination, and body weight at birth, which were recorded in the "Maternal and Child Health Handbook (Boshi-Kenko-Techo)", were collected. Laboratory data (HBsAg, HBeAg, anti-HBe, HBV-DNA, AST, and ALT), and clinical diagnosis of the mothers were also investigated. The period of sample collection for this study was between April 2018 and December 2019. The protocol was aligned with the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the Institutional Ethics Committee of Ehime Prefectural Central Hospital, Ehime, Japan.

Subjects

The subjects comprised females chronically infected with HBV genotype D and their children, who had a history of HB vaccination, 3 times, as recommended by the nationwide mother-to-child transmission prevention project. Mothers who had been followed up by our hospital once to several times per year due to chronic HBV infection were selected. The purpose of the present study was explained to them, and 17 mothers (37.4±3.9 years old) agreed to participate. Twenty-five children born to the participating mothers (7 males, 18 females; 8.5±4.1 years old) visited our hospital thereafter. The purpose and methods of the study were explained to the children as well as the mothers, and written informed consent was obtained from all mothers and any children over the age of 15.

The body weight at birth of all the children was greater than 2,000 g, and all children received 3 inoculations with HB vaccines between March 2000 and December 2015.

Clinical and laboratory data

Age, sex, and serum levels of HBsAg, anti-HBs antibody (anti-HBs), and anti-hepatitis B core antibody (anti-HBc) of the children were studied. Age, diagnosis, HBsAg, HBe antigen, and antibody (HBeAg/anti-HBe), HBV-DNA, AST, and ALT serum levels of the mothers were also investigated. The HBV genotype of mothers was determined to be D, using a commercially available kit (HBV genotype EIA, Institute of Immunology, Tokyo, Japan), according to the manufacturer's instructions. Mothers were diagnosed according to the criteria and definition of AASLD 2018 Hepatitis B Guidance (12).

Assay of HBV markers

HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc were assayed using chemiluminescent enzyme immunoassay (CLIA, ARCHITECT®, Abbott Japan, Tokyo, Japan). HBV-DNA was assayed by real-time polymerase chain reaction (PCR) (Cobas® 8800 HBV, Roche Diagnostics, Tokyo, Japan). Samples were assayed according to the manufacturer's protocols. The results of HBsAg, anti-HBs, and anti-HBc were expressed as IU/mL, mIU/mL, and S/CO (sample/cut-off), respectively, and values <0.05 IU/mL, <10.00 mIU/mL, and <1.00, respectively, were judged as negative. The results of HBeAg and anti-HBe were expressed as S/CO, and %, respectively, and values <1.00 and <50%, respectively, were judged as negative.

Table 1. Mothers' Laboratory Data.

No.	Age	Diagnosis*	HBsAg (IU/mL)	HBeAg (S/CO)	anti-HBe (%)	HBV DNA (log IU/mL)	AST (U/L)	ALT (U/L)
mother 1	33	CHB	44,096	(-)	99.9	3.5	18	20
mother 2	36	CHB	931	(-)	99.0	3.6	20	22
mother 3	34	CHB	1,218	(-)	99.0	3.6	19	9
mother 4	38	CHB	18,933	(-)	96.0	3.4	17	20
mother 5**	43	a-CHB	13,483	(-)	99.0	7.9	44	51
mother 6	34	i-CHB	4,989	(-)	99.7	<1.8+	17	14
mother 7	38	i-CHB	81.6	(-)	99.2	2.6	20	15
mother 8	33	i-CHB	5,623	(-)	99.2	2.8	15	14
mother 9	31	i-CHB	2,281	(-)	99.3	2.4	16	15
mother 10	40	CHB	470	(-)	99.0	3.3	19	14
mother 11	35	CHB	1,556	(-)	99.7	3.8	19	22
mother 12	40	CHB	1,752	(-)	99.6	4.2	25	16
mother 13	41	CHB	9,913	(-)	99.5	3.4	23	18
mother 14	41	i-CHB	118	(-)	99.0	2.9	16	17
mother 15	34	i-CHB	601	(-)	99.0	2.8	18	14
mother 16	40	i-CHB	1,850	(-)	99.0	2.4	16	17
mother 17	44	CHB	3,681	(-)	99.5	3.4	23	18

*CHB: chronic hepatitis B, a-CHB: immune-active chronic hepatitis B, i-CHB: inactive CHB, HBV: hepatitis B virus, AST: aspartate aminotransferase, ALT: alanine aminotransferase

**Laboratory data prior to the start of entecavir treatment. At the time of delivery, entecavir treatment had not been started.

Results

Clinical data of mothers

All mothers were positive for HBsAg (6,563±11,005 IU/mL), negative for HBeAg, and positive for anti-HBe (Table 1). The HBV-DNA levels were <3.3 log IU/mL (<2,000 IU/mL) in 7, 3.3-4.3 log IU/mL (2,000≤ ≤20,000 IU/mL) in 9, and >4.3 log IU/mL (>20,000 IU/mL) in one mother. The levels of AST and ALT were less than 30 U/L in all mothers except one. One mother was diagnosed with immune-active chronic hepatitis B (CHB), while inactive CHB was observed in seven mothers. The remaining nine diagnosed with CHB fulfilled the criteria of inactive CHB, except for the level of HBV-DNA. A mother with immune-active CHB (mother 5) had been treated with entecavir, but the therapy was started after delivery of the child that was included in this study. The other 16 mothers had no history of anti-viral therapy against CHB.

Types of vaccine and viral markers in children

The types of vaccines and viral markers of the children are shown in Table 2. Among the 25 children, 20 were vaccinated with a genotype C-derived vaccine (Bimmugen®, KM Biologics, Kumamoto, Japan), one with a genotype A-derived vaccine (Heptavax®-II), and one was vaccinated with both a genotype A-derived vaccine twice and a genotype C-derived vaccine once. The type of vaccine was undetermined in the remaining three children, as the type of HB vaccine was not recorded in their Maternal and Child Health Handbook.

As shown in Table 2, all 25 children were negative for HBsAg and anti-HBc, and 13 (52.0%) were positive for anti-HBs. Among the 20 children inoculated with only the genotype C-derived vaccine, all were negative for both HBsAg and anti-HBc, 11 of them (55.0%) were positive for anti-HBs. Regardless of the type of vaccine, mother-to-child transmission was prevented in all subjects. The relationship between positivity or anti-HBs titers and type of vaccines could not be analyzed as the sample size was too small.

Discussion

Epidemiological data on the decrease in HBV carriers in many countries, as well as the decreased risk of incidence of hepatocellular carcinoma, after the introduction of universal vaccination indicates that HB vaccines are effective at preventing the transmission of different HBV genotypes (1, 18). Genotype A-derived vaccines are used in the majority of countries worldwide; epidemiological data on the reduction of HBV carrier rates in these countries as well as experimental data using serum samples of subjects immunized with the HB vaccine indicate, that genotype A-derived vaccines are effective at preventing the transmission of other genotypes (1, 2, 9, 19). In Japan, genotype C- and genotype A-derived vaccines are used in the nationwide project for mother-to-child transmission prevention. Based on epidemiological data, after the introduction of this project, regarding the decreased rate of HBV carriers and the decreased incidence of hepatocellular carcinoma in young adults, it is suspected that these two types of vaccines are effective at pre-

Table 2. Children's HBV Markers.

No.	Mother	Age	Sex	Genotype of vaccine	HBsAg (IU/mL)	anti-HBs (mIU/mL)	anti-HBc (S/CO)
Child 1	mother 1	12	F	C	(-)	(-)	(-)
Child 2	mother 1	10	F	C	(-)	(-)	(-)
Child 3	mother 2	12	M	C	(-)	(-)	(-)
Child 4	mother 2	10	F	C	(-)	44.1	(-)
Child 5	mother 2	4	F	C	(-)	44.5	(-)
Child 6	mother 3	10	M	C	(-)	129.6	(-)
Child 7	mother 3	8	F	C	(-)	58.3	(-)
Child 8	mother 4	5	M	C	(-)	11.4	(-)
Child 9	mother 5	8	F	C	(-)	(-)	(-)
Child 10	mother 5	6	F	C	(-)	(-)	(-)
Child 11	mother 6	11	F	C	(-)	(-)	(-)
Child 12	mother 6	9	F	C	(-)	(-)	(-)
Child 13	mother 7	6	M	C	(-)	295.0	(-)
Child 14	mother 8	3	F	C	(-)	1,310	(-)
Child 15	mother 9	3	F	C	(-)	304.0	(-)
Child 16	mother 10	10	F	C	(-)	(-)	(-)
Child 17	mother 11	3	M	C	(-)	(-)	(-)
Child 18	mother 12	9	F	C	(-)	36.6	(-)
Child 19	mother 12	7	F	C	(-)	61.0	(-)
Child 20	mother 13	3	M	C	(-)	111.9	(-)
Child 21	mother 3	6	F	A	(-)	15.9	(-)
Child 22	mother 14	15	F	A+C*	(-)	(-)	(-)
Child 23	mother 15	10	F	?	(-)	243.0	(-)
Child 24	mother 16	14	F	?	(-)	(-)	(-)
Child 25	mother 17	19	M	?	(-)	(-)	(-)

*genotype A-derived vaccine 2 twice, genotype C-derived vaccine once

M: male, F: female

?: The type of vaccine could not be determined.

venting the transmission of genotypes B and C, the domestic genotypes in Japan (5, 6, 20). However, the above epidemiological data is insufficient to clarify whether the genotype C-derived vaccine is effective at preventing transmission of genotype D, as genotype D is a minor genotype in Japan, and there is currently no published research regarding the effect of the genotype C-derived vaccine on the prevention of mother-to-child transmission to children born to mothers infected with genotype C. Moreover, reports were scarcely found regarding the efficacy of universal vaccination using genotype-C-derived vaccines in areas or countries where genotype D is circulating predominantly (21). In this context, we attempted to clarify this issue.

It is reported that the risk of acquiring HBV infection in infants born to mothers with both HBsAg and HBeAg is very high (70-100%) without vaccination, whereas those born to mothers with HBsAg and without HBeAg are 5-30% in Asia (3). In the present study, all 20 children born inoculated with the genotype C-derived vaccine were negative for both HBsAg and anti-HBc. Although, in this study, the majority of mothers were negative for HBeAg, the present data indicates that the genotype C-derived HB vaccine is effective at preventing HBV transmission in children born to mothers infected with HBV genotype D.

In the present study, anti-HBs was positive in 13/25 children with a mean age of 8.5±4.1 years, and in 11/20 children inoculated with the genotype C-derived vaccine. Therefore, approximately half of the subjects were negative for anti-HBs. It is of great concern whether administration of HB-Ig followed by the inoculation of genotype C-derived vaccine to infants born to mothers infected with genotype D is effective enough to induce, and maintain an anti-HBs-positive state for a long time. There are 2 possibilities regarding the negative result of anti-HBs in inoculated children; one is that they were not actively immunized, and the other was that they had become positive for anti-HBs and the titer of anti-HBs declined to a negative level. In general, more than 95% of infants are actively immunized against HBsAg who are vaccinated with the HB vaccine, regardless of the administration of HB-Ig (1, 4). Moreover, it is well known that the titer of anti-HBs increases several weeks after the third inoculation, and its titer thereafter gradually declines. Nommensen et al. reported that the half-life of anti-HBs was 40-280 days (mean 150; SD 40) (22). It has also been reported that approximately 1/3 to 1/2 of those inoculated became negative for anti-HBs four years after vaccination (23). Another report showed that anti-HBs became undetectable in 15-50% of children 5-15 years after vaccina-

tion, as opposed to in 30-60% of adults within 5 years (24). In the present study, anti-HBs data several weeks after the third inoculation could not be obtained; however, it is suspected that the majority of the subjects negative for anti-HBs were actively immunized after vaccination, and the titer of anti-HBs gradually declined to levels <10 mIU/mL thereafter.

Although there are not many reports describing the positive rate of anti-HBs in children of each age after the inoculation of HB vaccine in infants, a report from Taiwan after the national project of universal vaccination indicated that the positive rate of anti-HBs at the age of 7-8 was approximately 50% (25). Therefore, the positive rate of anti-HBs in the present study may not be low.

Another problem is whether there is a difference between the rate of anti-HBs in children inoculated with genotype C-derived vaccine born to mothers infected with genotype C, and those infected with genotype D. No study has investigated this issue, but there was a report from Japan related to this issue; family members (including adults) of HBV carriers with HBsAg subtype *adr* (supposed to be genotype C) and those with subtype *ayw* (supposed to be genotype D) were inoculated with plasma-derived HB vaccine (mainly consisting of subtype *adr* HBsAg), and HBsAg/anti-HBs were studied at 1 month and 5 years after inoculation (26). No subjects were positive for HBsAg, and positivity for anti-HBs at 1 month after inoculation were 91% and 83%, respectively; and at 5 years post inoculation positivity for anti-HBs were 73% and 71%, respectively. This study indicates that there is no difference in the positivity of anti-HBs after inoculation with genotype C-derived vaccine between family members of genotype C carriers and those of genotype D carriers. However, further investigation is needed to answer this question.

Kato et al. studied the effect of vaccine-acquired polyclonal anti-HBs on the prevention of HBV in non-vaccine genotypes (9), and found that vaccination with genotype A- or C-derived HBsAg provided polyclonal anti-HBs that were sufficiently capable of binding to HBsAg of non-vaccine genotypes; however, a small portion of anti-HBs were specific to the vaccine genotype HBsAg. Thus, they concluded that high titers of anti-HBs would be required to prevent non-vaccine genotype HBV infection. In the present study, the preventative capabilities of genotype-C-derived vaccines against genotype D transmission were satisfactory, at least during childhood. However, the mean age of the children in this study was 8.5 years; therefore, we are not able to speculate whether anti-HBs acquired by genotype C-derived vaccination can prevent the transmission of genotype D HBV during adult life as well. Hence, further studies are necessary to clarify the long-term effects of this vaccination regimen. Moreover, studies regarding the efficacy of genotype C-derived vaccination against genotype A transmission are scarce (9, 27); this problem should also be clarified in future studies.

Conclusion

All children born to mothers chronically infected with genotype D and vaccinated with the genotype C- and/or genotype A-derived vaccines were negative for both HBsAg and anti-HBc. The results of the present study suggest that the genotype C-derived HB vaccine is effective for preventing HBV transmission in children born to mothers infected with HBV genotype D. These results have significant translational implications for preventing HB transmission from mothers to children, using the genotype C-derived vaccines.

The authors state that they have no Conflict of Interest (COI).

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