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Immunogenicity and Efficacy of a Recombinant DNA Hepatitis B Vaccine, GenHevac B Pasteur in High Risk Neonates, School Children and Healthy Adults

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SUMMARY **The immunogenicity and the protective efficacy of a recombinant DNA hepatitis B vaccine, GenHevac B Pasteur with or without passive immunization with hepatitis B immunoglobulin (HBIG) in high risk neonates born from HBsAg and HBeAg positive mothers was evaluated. Twenty-six neonates (group A) received HBIG 100 IU intramuscularly at birth plus GenHevac B Pasteur 20 μg at birth, 1, 2 and 12 months of age while another 23 neonates (group B) received only GenHevac B Pasteur vaccine. Forty high risk newborns who received no immunization served as control group. It was found that at months 4, 12, 13 and 24 the seroconversion rate in both group A and B were very high in the range of 95-100% with the GMT ranging from 10-160,000 mIU/ml. In the control group of infants, 85% had HBsAg positive at one year of age but it was only 3.8% and 8.7% in vaccinated groups A and B, respectively. The protective efficacy in neonates group A and B were 95.5% and 89.8% at one year, respectively, with no statistically significant difference.**

In 46 normal school children (group C) and 48 healthy adults (group D) who received the same dose of GenHevac B Pasteur the seroconversion rates at month 4 after receiving 3 doses of vaccination were 97.8% and 83.3% in group C and group D, respectively. At month 12, the seroconversion rate in group C rose to 100% and was significantly higher than the 89.6% of group D. However, at month 13, after a booster dose at month 12, the seroconversion rate of group D also rose to 95.8%, close to the 100% of group C. The GMT of anti-HBs responses in school children were statistically significant higher than those of healthy adults at months 4, 6 and 12, but both groups showed similar anamnestic antibody responses after the booster dose at month 12 with a GMT of 27,800 mIU/ml and 12,520 mIU/ml in groups C and D, respectively. This full dose of GenHevac B Pasteur produces high immunogenicity and protective efficacy in high risk neonates as well as in school children and healthy adults. During the first year the seroconversion rate in healthy adults was lower than that of school children but finally catched up at month 13 with high GMT levels. It is concluded that full dose recombinant DNA hepatitis B vaccine alone is equally effective as the vaccine plus HBIG in prevention of vertical HBV transmission from HBeAg carrier mothers to their high risk neonates.

Hepatitis B virus (HBV) infection is one of the major health problem in many parts of the world.[1] Most chronic HBV carriers develop chronic hepatitis and progressive liver destruction leading to macronodular cirrhosis and may lead to the development of hepatocellular carcinoma which is one of the most common cancers in Southeast Asia. In hyperendemic areas such as Asia and Oceania, HBV is transmitted from asymptomatic carrier mothers to their babies, especially when the mothers are seropositive for hepatitis-B e-antigen.[2-4] Almost all of such babies become persistent HBsAg carriers and constitute a reservior of HBV for further horizontal and later in life, vertical spreads in the community.[5-8] The newborn is also particularly vulnerable because the carrier mother is often highly infectious during the process of labour and delivery. Although HBV transmission also can occur under other circumstances, infection from mother to infant is the major driving force maintaining high HBV carrier rates in many parts of the world and especially in Thailand.[9-11] Fortunately, mother to infant transmission rarely occurs in utero and immunoprophylaxis administered to the high risk newborns shortly after birth can significantly prevent almost all of the maternal transmission leading to the carrier status in their in-

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fants.[8,12-17] A recombinant hepatitis B vaccine, GenHevac B Pasteur, containing pre S_1, pre S_2, and S proteins produced in mammalian cells has been recently developed by Pasteur Vaccins and was licensed in France in December 1987. The characteristics of GenHevac B necessitate an evaluation of the immunogenicity of the vaccine for the anti-HBs antibody response in both children and adults. This vaccine should be of great interest in the high risk newborns to highly infective mothers for preventing HBV perinatal transmission. The objective of the present study was to evaluate the tolerance, immunogenicity and protective efficacy of the recombinant DNA hepatitis B vaccine, GenHevac B Pasteur, 20 μg per dose, in high risk neonates born to chronic HBV carrier mothers as combined passive-active immunoprophylaxis or as active immunization alone. The immunogenicity of this vaccine in normal school children and adults was also evaluated.

MATERIALS AND METHODS

Immunogenicity and protective efficacy of the vaccine in high risk neonates

GenHevac B Pasteur, 20 μg per dose injection in high risk neonates born to HBsAg and HBeAg positive mothers was studied. From June to December 1988, sixty pairs of asymptomatic pregnant Thai women were selected for a protective study of vaccination against perinatal HBV transmission. Initial screening of pregnant women for HBsAg and HBeAg was done by reverse passive hemagglutination (RPHA). All newborn infants of the e-antigen positive carrier mothers were normal full term neonates with birth weight over 2,500 g. Inform consent was obtained from the parents before giving vaccination. These newborns were randomly allocated into two groups, A and B (30 infants in each group) according to the random permutation table constructed by randomization prior to study. Newborns of group A received hepatitis B immunoglobulin (HBIG) 100 I.U. intramuscularly at the antero-lateral aspect of thigh within 24 hours after birth and GenHevac B pasteur 20 μg intramuscularly at birth, 1, 2 and 12 months of age. Newborns of group B received GenHevac B Pasteur 20 μg intramuscularly at the antero-lateral aspect of thigh alone at birth, 1, 2 and 12 months of age. The follow up for clinical evidence of hepatitis or complications of vaccination and for serological tests of hepatitis B markers as well as antibody response was carried out at 2, 4, 12, 13 and 24 months of age. All infants' serum samples were tested for HBsAg, anti-HBs and anti-HBc by the enzyme linked immunosorbent assay (ELISA).

For the comparison of the effectiveness of vaccination, 40 infants born from HBeAg positive/HBsAg carrier mothers who refused to receive hepatitis B vaccination were followed up at one and two years of age for the incidence of HBV transmission by the determination of HBsAg. In this control group, 34 out of 40 (85%) infants became HBsAg positive at one and two years of age. The protective efficacy of hepatitis B vaccination was calculated as previously described.[18]

Immunogenicity of the recombinant DNA vaccine in normal children and adults

From June 1988 to October 1989, the immunogenicity of GenHevac B Pasteur was evaluated in normal school children and healthy adults. The initial screenings of school children and healthy adults for HBsAg, anti-HBs and anti-HBc were done by ELISA. Forty-six normal school children aged 4 to 14 years (group C) and forty-eight healthy adults aged 15 to 41 years (groups D) who were negative for all hepatitis B markers and had no history of receiving HBIG within 6 months were immunized with GenHevac B Pasteur 20 μg per dose intramuscularly in the deltoid, three injections one month apart and one booster dose at one year after the first injection. The follow up for clinical evidence of hepatitis or side effects of vaccination and for serological test of hepatitis B markers were also carried out at 2, 4, 6, 12 and 13 months after first dose of vaccination. All serum samples were tested for HBsAg, anti-HBs and anti-HBc by ELISA.

Laboratory methods

The laboratory tests for hepatitis B markers were performed as follows. Initial screening of pregnant women for HBsAg and HBeAg was done by the reversed passive hemagglutination method (RPHA) using Anti-Hebscell Neo and Anti-e cell Neo (The Green Cross Corporation, Osaka, Japan). The blood specimens from the HBeAg positive carrier mothers were confirmed once more after delivery. The specimens from infants receiving vaccination (group A and group B), from school children (group C) and from healthy adults (group D) were tested for HBsAg, anti-HBs and anti-HBc by enzyme linked immunosorbent assay (ELISA) using Monolisa® (Diagnostic Pasteur, France). The Monolisa® anti-HBs sensitivity is 5 mIU/ml and the protective level is equal to or higher than 10 mIU/ml.

Hepatitis B vaccine and HBIG

The recombinant DNA hepatitis B vaccine, GenHevac B Pasteur, Lot No. 92367 was obtained from Pasteur Vaccins, France. The vaccine was prepared in single dose syringe, ready for use, containing 20 μg/ml/dose of purified and inactivated HBsAg, aluminium hydroxide as adjuvant (maximum 1.25 mg of aluminium) and 0.5 ml of excipient. The HBIG used in this study was prepared by French International

Blood Center, Lot No. 76F10 in keeping with the European Pharmacopia requirements. One millilitre dose contained 100 IU.

Statistical methods

The statistical analysis was performed by student's *t*-test and by chi-square test when appropriate.

RESULTS

The side effects of vaccination

There were no serious reactions to the vaccine and the adverse effects observed were mild and transient. Only 2.1% of adults had local reactions for 2-3 days as soreness, redness and erythema (Table 1). Local swelling and erythema were observed in 3.3% of neonates. General reactions, including headache and fever lasting for 1-2 days, occurred in 3-4% of both children and adults.

Immunogenicity of the vaccine in neonates

Among 30 high risk neonates in each group A and B, only those who had perfectly followed up to 1 year were evaluated (26 in group A and 23 in group B). The results obtained after the vaccination of the 26 high risk neonates who received GenHevac B vaccine plus HBIG (Group A) and of the 23 high risk neonates who received GenHevac B vaccine alone (Group B) are shown in Table 2, and Figure 1. The seroconversions at month 4 and month 12 were similar in both groups approaching 95-96% and rose up to 100% at 13 months in group A while it was 95.2% in group B. However, there was no statistically significant difference in the seroconversion rate between these 2 groups ($P > 0.05$). The geometric mean titers (GMT) at month 4 and month 12 were also similar between the two groups. After a booster dose of vaccination at 12 months of age, the GMT rose significantly in both groups, reaching 7,985 mIU/ml and 4,542 mIU/ml one month later in group A and group B, respectively. The antibody titer declined gradually resulted in the GMT titer of 627 mIU/ml in group

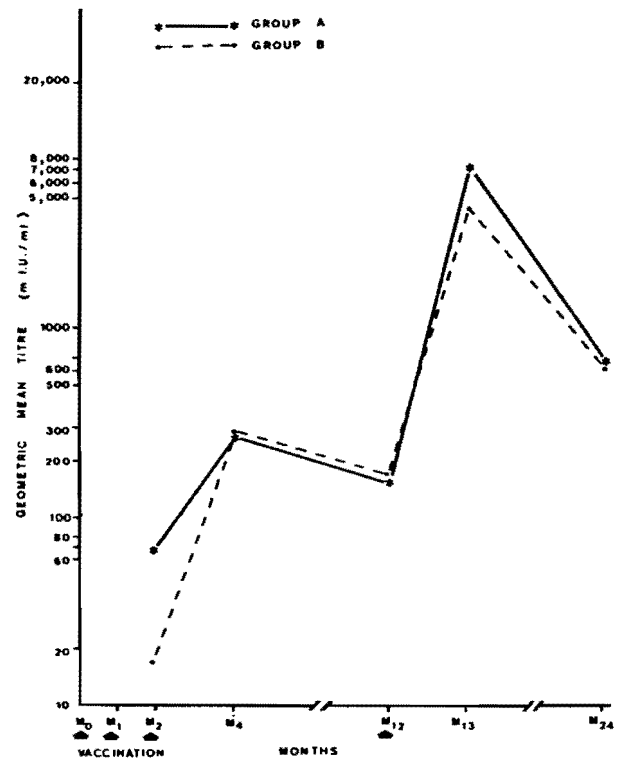

Fig. 1 The antibody response following 20μ g GenHevac B Pasteur injection in newborn infants group A and group B

Table 1 Frequency of side effects in three groups of vaccinees after vaccination with GenHevac B Pasteur

Side effects	High risk neonates (N = 49)	School children (N = 46)	Adults (N = 48)
Local reactions			
Soreness (%)	0	0	2.1
Redness (%)	0	0	2.1
Erythema (%)	3.3	0	2.1
General reactions			
Fever (%)	3.3	4.3	4.1

Table 2 The seroconversion rate (%) and the geometric mean titer (GMT) of the anti-HBs response after injection of HBIG and GenHevac B Pasteur in group A and GenHevac B Pasteur alone in group B of high risk neonates

Study group	Evaluation	M2	M4	M12	M13	M24
A	Seroconversion# rate (%)	100	96	96	100	95.8
	GMT (mIU/ml)	67.4	277.2	161.4	7,985.0	627.0
	(range)	(10-550)	(42-3,000)	(10-4,000)	(20-160,000)	(33-25,000)
	Number	26	26	26	26	25
B	Seroconversion# rate (%)	61.9*	95.2	95.2	95.7	94.7
	GMT (mIU/ml)	18.0	279.0	186.0	4,542.0	607.0
	(range)	(10-880)	(10-4,200)	(10-5,000)	(10-128,000)	(10-30,000)
	Number	23	23	23	23	22

* Significant different from group A at M2 ($p<0.05$)

= $\frac{\text{No of anti HBs positive} \geq 10 \text{ mIU/ml}}{\text{No tested}} \times 100$

Table 3 Protective efficacy of immunoprophylaxis in high risk neonates either with the combination of HBIG and GenHevac B Pasteur (group A) or GenHevac B Pasteur vaccination alone (group B)

Study group	Evaluations	M12	M24
A	HBsAg positive (%)	3.8	4.0
	Protective efficacy (%)	95.5	95.3
	Anti-HBc positive (%)	38.5	16.0
	Number	26	25
B	HBsAg positive (%)	8.7	13.6
	Protective efficacy (%)	89.8	84.0
	Anti-HBc positive (%)	47.8	22.7
	Number	23	22
Control	HBsAg positive (%)	85	85
	Number	40	40

A and 607 mIU/ml in group B at the age of 24 months (Fig. 1). The majority of infants still had antibody titer above the protective level (10 mIU/ml) at 2 years of age.

Protective efficacy

The protective efficacy of the immunoprophylaxis was determined by comparison of the incidence of HBV infection as shown by positive HBsAg in the vaccinated group and non-vaccinated group as previously discribed.[18] The results are shown in Table 3. The protective efficacy in group A and group B at 12 months of age was 95.5% and 89.8%, respectively, which was not significantly different.

Immunogenicity of vaccine in school children and adults

The results obtained after the immunization with GenHevac B Pasteur in 46 normal school children and 48 healthy adults are shown in Table 4 and Fig. 2. the seroconversion rates at month 4 after receiving 3 doses of vaccination were 97.8% in school children and 83.3% in healthy adults. This value reached 100% in school children at month 12, which was significantly higher than that in adults (89.6%, $p < 0.05$). The GMT of anti-HBs response in school children were also significantly higher

Table 4 The incidence of the seroconversion rate (%), the geometric mean titers (GMT) of anti-HBs response after immunization with GenHevac B Pasteur in healthy school children (group C) and adults (group D)

Study group	Evaluation	M2	M4	M6	M12	M13
C (N = 46)	Seroconversion# rate (%)	78.3	97.8	97.8	100	100.0
	GMT (mIU/ml) (range)	39.0 (10-1,300)	726.0 (10-5,000)	1,015.0 (80-12,000)	571.4 (10-3,600)	27,800.0 (65-353,000)
	Anti HBc + ve	–	–	–	0	0
D (N = 48)	Seroconversion# rate (%)	60.4	83.3*	89.6	89.6*	95.8
	GMT (mIU/ml) (range)	11.3 (10-1,100)	146.0† (10-4,600)	245.1† (10-3,800)	114.8† (10-3,700)	12,520.0 (70-325,000)
	(Anti HBc + ve	–	–	–	0	0

† Significant different from school children at M4, M6, M12 ($P<0.05$)

* Significant different from school children at M4 and M12 ($P<0.05$)

= $\frac{\text{No of antiHBs Positive} \geqslant 10 \text{ mIU/ml}}{\text{No tested}} \times 100$

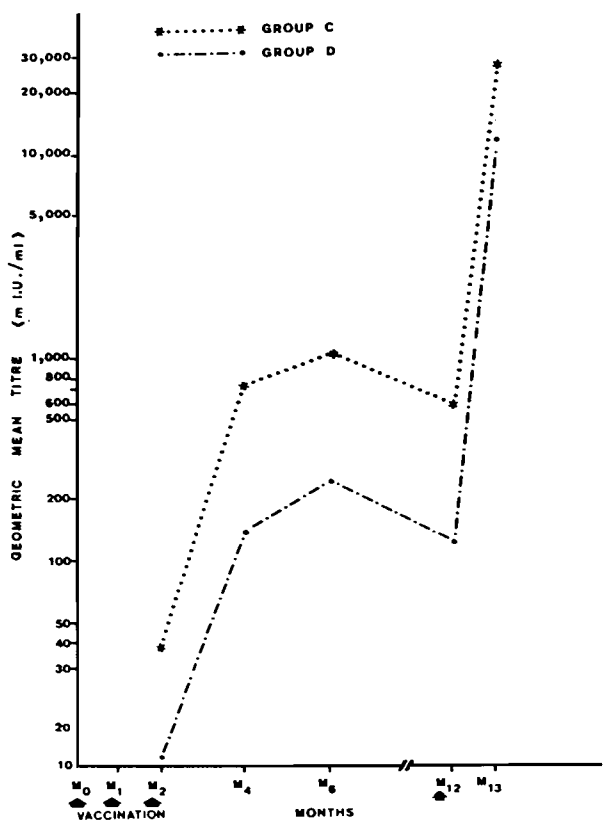

Fig. 2 The antibody response following 20μg GenHevac B Pasteur injection in school children (group C) and healthy adults (group D)

than healthy adults ($p < 0.05$) at months 4, 6 and 12 (Fig. 2). After a booster dose at month 12, both school children and adults showed anamnestic antibody resposne with the GMT rose up to 27,800 mIU/ml in school children and 12,520 mIU/ml in adults one month later.

DISCUSSION

Hepatitis B virus infection is common in Thailand and Southeast Asia as a whole. Approximately 10% of Thai children of school age become chronic carrier for HBsAg mainly from vertical transmission of HBV from their HBsAg carrier mothers. In the endemic area where perinatal transmission of HBV is the main mode of infection, it has been shown that immunization with hepatitis B vaccine will prevent the development of the infection in 75-80% of infants born to highly infectious HBeAg-positive mothers. Concurrent use of hepatitis B immunoglobulin (HBIG) and hepatitis B vaccine in such infants appears to increase the rate of protection to as high as 95%.[2,12,14] However, the price of HBIG is rather expensive

in those developing countries and prevent its use in the nationwide immunization program. Attention has been focused to the use of only hepatitis B vaccine for perinatal immunoprophylaxis to prevent HBV infection in the neonates. An approach using a recombinant hepatitis B vaccine alone in the full dose of 10 μg given at birth, 1, 2 and 12 months of age revealed a protective efficacy of 94.5% in high-risk infants, the majority of whom had relatively high titers of anti-HBs antibody at 13 months of age.[19] In the present study, the use of full dose of a recombinant DNA vaccine, GenHevac B Pasteur, given alone at birth, 1, 2 and 12 months of age resulted in seroconversion rates and protective efficacy comparable with those obtained by the use of vaccine combined with HBIG. Although the protective efficacy in the high risk neonates receiving vaccine alone (89.8% at 1 year, and 84.0% at 2 years of age) was slightly lower than the high risk neonates who received the combination of vaccine and HBIG (95.5% at 1 year and 95.3% at 2 years of age), there was no statistically significant difference between these two groups ($p > 0.05$). Furthermore, the seroconversion rate and antibody titers were comparable in both groups at one and two years of age. The majority of the vaccinees still had antibody titers above the protective level (10 mIU/ml) at 2 years of age. This study confirmed that the recombinant DNA hepatitis B vaccine can be used alone to effectively prevent HBV transmission in the neonates from their HBsAg carrier mothers. The vaccine can be produced in large quantity and the price will eventually be reduced, to replace the expensive plasma-derived vaccine. In fact the efficacy of the recombinant DNA vaccine was proved to be comparable with the plasma derived vaccine in perinatal immunization in high risk neonates born from HBsAg/HBeAg carrier mothers.[15] Other factors influencing the efficacy of perinatal immunization are the schedule of immunization and the immunogenicity of the vaccine itself. It has been shown that for individuals at high risk of hepatitis B infection, vaccination at months 0, 1, 2 and 12 might be considered for obtaining an optimal early seroconversion as well as long-term protection.[20,21] Early development of anti-HBs antibody in the majority of high risk neonates is required for adequate post-exposure immunization. In this study, when using vaccine alone 95.2% of infants had seroconversion after three doses of vaccination at 4 months of age with mean GMT of 279.0 mIU/ml indicated that the vaccine is highly immunogenic and the effective protection is possible. The development of GenHevac B vaccine to contain not only the S antigen but also pre-S_1 and pre-S_2 regions is probably results in high immunogenicity. In the previous study, a rapid increase of anti-pre S_2 antibody was observed within 4 months after administration of three doses of plasma derived hepatitis B vaccine (Hevac B Pasteur).[22] This early rise of anti-pre S_2 antibody could be beneficial for high risk infants by preventing the attack and replication of HBV in hepatocytes.

Another very important problem in the control, and especially the elimination of hepatitis B virus is the duration of immunity and the necessity for booster immunization. It is generally accepted that immunity to hepatitis B persists for as long as at least 10 mIU/ml of anti-HBs can be detected in the serum. For infants born to mothers who are HBV carriers, a 7 year study in Senegal has demonstrated that protection against viraemic HBV infection can decline within 6 years of age. It was previously concluded that children in endemic areas should be given a booster dose five years after the initial vaccination.[23] In the present study, although the GMT of antibody titer declined from 4,542 mIU/ml at 13 months of age (after a booster dose at 12 months) to 607 mIU/ml at 2 years of age, the seroconversion rate was not significantly decreased (95.2% at 12 months of age as compared with 94.7% at 2 years). The majority of children still had relatively high titers of anti-HBs at 2 years of age, ie above 100 mIU/ml in 82.5% of group A, and 85.0% of group B. The declining pattern of the antibody levels in these children is expected to be the same as previous reports. Although it may not be necessary to give a booster dose at 5 years as previously recommended, we suggest re-vaccination of children in the school age and adults in endemic areas to obtain the best result of HBV eradication. The excellent antibody response to the recombinant DNA hepatitis B vaccine in school age children as shown in this study would ensure the successful immunization program. The strategy for the control of hepatitis B in countries with hyperendemic HBV infection where most infections are acquired early in life, the vaccine should be administered shortly after birth to all infants for populations with carrier rates 10%. It was stressed that when adequate quantities of hepatitis B vaccine are available, immunization campaigns should be considered for incorporation into the WHO Expanded Program on Immunization (EPI).[24]

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