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Immunogenicity and Protective Efficacy of Low Dose Recombinant DNA Hepatitis B Vaccine in Normal and High-Risk Neonates

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SUMMARY **A low dose of recombinant DNA hepatitis B vaccine (HB-VAX II® , MSD) was tested for efficacy in the prevention of perinatal hepatitis B virus (HBV) transmission in normal and high-risk neonates born from HBsAg carrier mothers. A dose of 2.5 μg recombinant vaccine was injected intramuscularly at 0, 1, 2 and 12 months of age to 30 newborns from HBsAg negative mother (group I), 30 from HBeAg negative/HBsAg carrier mother (group II) and 30 from HBeAg positive/HBsAg carrier mother (group III). The incidence of persistent HBsAg carrier infants at 13 months of age was 0, 0, and 30.4 percent in groups I, II and III, respectively. The protective efficacy in high risk infants in group III was 65.7 percent. The seroconversion at month 4, after the third dose of vaccination was 96.3, 95.7 and 100 percent in group I, group II and group III, respectively. After a booster dose of vaccination at 12 months of age, the seroconversion rose to 100 percent at month 13 in all three groups. The geometric mean titer (GMT) of anti-HBs antibody at 13 months of age were 2,092, 1,657 and 1,938 mIU/ml in group I, group II and group III, respectively. It is concluded that the low dose (2.5 μg) recombinant hepatitis B vaccine using alone is effective in prevention of perinatal HBV transmission in low risk infants (groups I and II), but it is less effective in high risk infants (group III). The low dose hepatitis B vaccine regimen should be combined with HBIG given at birth for the full protection of perinatal HBV transmission in the high risk neonates born from the HBeAg positive/ HBsAg carrier mothers which comprised approximately 3 percent of all newborns in the endemic area. This approach would certainly reduce the cost of hepatitis B immunization and it could be applied economically for the national program of HBV transmission contorl in neonates.**

Hepatitis B virus infection is one of the major public health problems in Thailand. Approximately 5-10% of the general population in the whole country acquired chronic hepatitis B carrier states. In children, infection with this virus occurs by vertical transmission from the carrier mothers to their infants and by horizontal transmission from infected persons mainly the family members. The risk of hepatitis B infection in newborn infants born from hepatitis B carrier mothers is approximately 31%, and the chance of their siblings being infected is approximately 37%.[1-4] The risk is especially high, up to 70-90%, in infants whose mothers are both HBsAg and HBeAg positive.[4,5] Such high risk carrier mothers are likely to bear a new generation of HBsAg carriers, thus maintaining the high rate of HBsAg carriage in the population and eventually the development of carcinoma of the liver in later life.[6] In a hepatitis B virus endemic area such as Thailand where HBeAg positive/ HBsAg carrier mothers are prevalent, universal vaccination of newborns is the most cost-effective way to prevent chronic HBV infection.

We have previously studied several schedules of vaccination in neonates to prevent HBV transmission from HBeAg positive/HBsAg carrier mothers to their infants using either plasma derived vaccine or recombinant hepatitis B vaccine alone or in combination with hepatitis B immunoglobulin (HBIG).[2,7-12] The effectiveness of different vaccines and vaccination programs revealed a protective efficacy of approximately 70-90%. However, the costs of HBIG

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and of screening for maternal HBV markers have to be considered. One way apparently to cut costs is to reduce the vaccine dose below recommended levels. This approach may reduce the anti-HBs production and decrease the protection afforded to high risk infants, especially in endemic countries where the risk of perinatal infection is high. It has been shown that a reduced dose (2 μg) of plasma derived hepatitis B vaccine (Hevac B®) could be used effectively as the standard dose (5 μg) in the prevention of perinatal HBV transmission in high risk neonates born from HBeAg positive/ HBsAg carrier mothers.[11,13] Little information is available concerning the use of low dose recombinant hepatitis B vaccine in neonates. We have undertaken a study using a low dose (2.5 μg) of recombinant hepatitis B vaccine to determine the immunogenicity and the protective efficacy both in normal and high risk neonates in Thailand. This information will be valuable for planning of the nationwide vaccination programme in relation to cost-effectiveness.

MATERIALS AND METHODS

From February 1989 to September 1989, ninety pairs of asymptomatic pregnant HBsAg carriers and their infants were selected for a prospective study of vaccination against perinatal HBV transmission. Initial screening of pregnant women for HBsAg and HBeAg was done in the prenatal clinic by reverse passive haemagglutination (RPHA) using Anti-Hebscell Neo and Anti e-cell Neo (The Green Cross Corporation, Osaka, Japan) as previously described.[3] These mother-infant pairs were divided into 3 groups, 30 pairs in each group. Group I consisted of infants born from HBsAg negative mothers, group II consisted of infants born from HBeAg negative/HBsAg carrier mothers and group III consisted of infants born from HBeAg positive/HBsAg carrier mothers. All infants were normal full-term neonates with birth weight over 2500 g. Informed consent was obtained from the parents of all infants before giving vaccination. All neonates received yeast derived recombinant hepatitis B vaccine (HB-VAX II, MSD, U.S.A. lot No. G 4426 and H4007, 10 μg per ml) at the dose of 2.5 μg intramuscularly within 24 hours after birth, followed by HB-VAX II 2.5 μg at 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 markers was carried at 0, 4, 12 and 13 months of age. All infants' serum samples were tested for HBsAg, anti-HBs and anti-HBc by the enzyme linked immunosorbent assay (ELISA) using Monolisa® HBsAg, Monolisa® anti-HBs and Monolisa® anti-HBc respectively (Diagnostic Pasteur, France). The Monolisa® anti-HBs sensitivity is 5 mIU per ml and the protective level is equal to or higher than 10 mIU per ml. Those samples with positive HBsAg were further determined for HBeAg by RPHA method using Anti-e-cell Neo (The Green Cross Corporation, Osaka, Japan).

For the comparison of the effectiveness of vaccination, 116 infants who were born from HBsAg carrier mothers but received no hepatitis B vaccination were followed up to one year of age for the incidence of HBV transmission by the determination of HBsAg. In this group of control infants, 2 out of 76 (2.6%) infants born from HBeAg negative/ HBsAg carrier mothers became HBsAg positive and 34 out of 40 (85%) infants born from HBeAg positive/HBsAg carrier mothers became HBsAg positive. The protective efficacy of hepatitis B vaccination was calculated by :

$$\text{Protective efficacy} = \frac{[\text{Attack rate in nonvaccinated group} - \text{Attack rate in vaccinated group}] \times 100}{\text{Attack rate in nonvaccinated group}}$$

Statistical analysis was performed by student's *t* test.

RESULTS

Among 90 vaccinated infants in this study, 66 (73%) had perfect follow-up records up to 13 months of age. One infant in each of group II and group III was lost to follow-up early and they were excluded from the study. The results of vaccination are shown in Table 1. No infants on group I and group II had evidence of HBV infection up to 13 months of age, while those in group III HBsAg was detected in 19.2, 29.2 and 30.4% at the ages of 4, 12 and 13 months respectively. This resulted in a protective efficacy of 65.7% in group III at 13 months of age. The seroconversion rates for infants in each group were analyzed according to the serological status of their mothers at 4, 12 and 13 months after vaccination. At month 4, after the third vaccine dose, infants in all three groups had similar seroconversion rates ranging from 95.7 to 100%. The high percentage of seroconversion persisted up to one year of age. After a booster dose of vaccine at 12 months of age, the seroconversion rate reached 100% at month 13 in all three groups. The geometric mean titer (GMT) of anti-HBs antibody in these three groups of infants are shown in Table 2. The infected infants in group III were excluded from the calculation of GMT for antibody response. The GMT of anti-HBs antibody response in all three groups at various times after vaccination are also shown in Fig. 1. It can be seen that the GMT of anti-HBs antibody responses in vaccinated infants were similar in all three groups except at month 4, when the GMT in group II was significantly lower than in the other two groups. The majority of infants

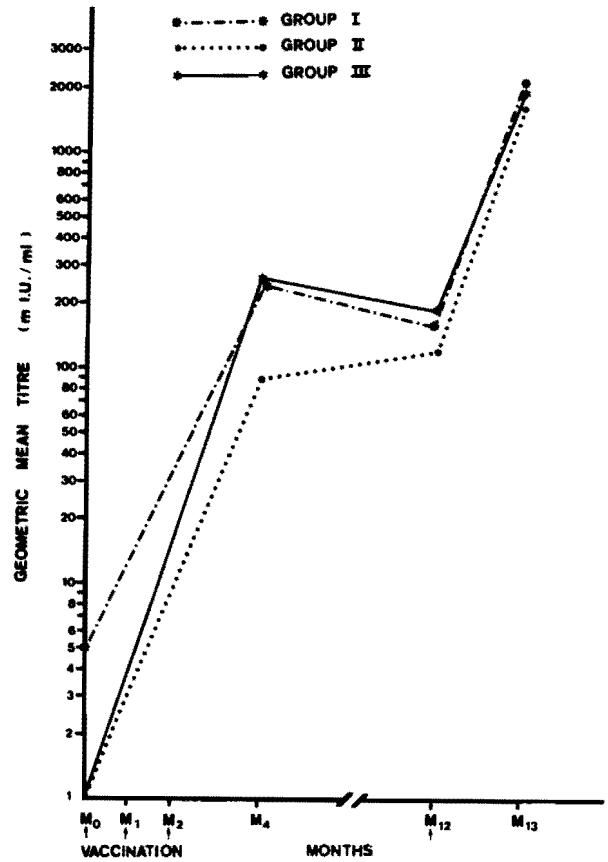

Fig. 1. The antibody response following 2.5 μg HBVAX II injection in newborn infants in groups I, II and III.

had anti-HBs antibody levels significantly higher than the protective level (10 mIU/ml) after the third dose of vaccination (at month 4) and it only slightly declined at 12 months of age. After a booster dose at month 12, the anti-HBs antibody rose to very high level in all three groups. The GMT of anti-HBs antibody at 13 months of age were 2,092, 1,657 and 1,938 mIU/ml in group I, group II and group III, respectively. There was no statistically significant difference in the antibody response among these three groups.

DISCUSSION

Without immunoprophylaxis, 70-90% of newborns of the HBeAg positive/HBsAg carrier mothers will become chronic carriers.[1,4,5] Our previous studies and several other studies have shown that significant decreases in the proportion of these infected neonates were obtained when hepatitis B vaccines were given either alone or in combination with HBIG.[2,7-19] The best prophylaxis schedule is the com-

Table 1. The incidence of HBsAg positive (%) and the seroconversion rate (%) in three groups of neonates receiving HBVAX II 2.5 μg at birth (M0), 1(M1), 2(M2) and 12(M12) months of age.

Study group	HBsAg^{+ve} (%)				Seroconversion rate # (%) at the age of			
	M0	M4	M12	M13	M0	M4	M12	M13
I Normal Infants from HBsAg⊖ mothers n=30	0 (0/30)	0 (0/27)	0 (0/25)	0 (0/23)	36.7 (11/30)	96.3 (26/27)	92.0 (23/25)	100 (23/23)
II Infants from HBsAg⊕, HBe Ag⊖ mothers n=29	0 (0/29)	0 (0/23)	0 (0/22)	0 (0/20)	0 (0/29)	95.7 (22/23)	95.5 (21/22)	100 (20/20)
III Infants from HBsAg⊕, HBe Ag⊕ mothers n=29	0 (0/29)	19.2 (5/26)	29.2 (7/24)	30.4 (7/23)	0 (0/29)	100 (19/19)	88.2 (15/17)	100 (16/16)

#Seroconversion rate (%) = $\frac{\text{No of AntiHBs Positive} \times 100}{\text{No of tested}}$

Table 2. GMT# of antibody response in three study groups following immunization at birth (M0), 1(M1), 2(M2) and 12(M12) months of age with HBVAX II 2.5 μg.

Study group	Type	Geometric mean titre and range of antiHBs (mIU/ml)			
		M0	M4	M12	M13
I	GMT#	5.0	253	158	2,092
Normal Infants	range	10–3,800	10–4,800	20–3,100	400–15,000
from HBsAg⊖ mothers	N	30	27	25	23
II	GMT#	1.0	93*	125	1,657
Infants from	range	–	10–980	10–600	32–24,000
HBsAg⊕, HBe Ag⊖ mothers	N	29	23	22	20
III	GMT#	1.0	266	187.9	1,938
Infants from	range	–	30–10,000	40–6,000	160–12,000
HBsAg⊕, HBe Ag⊕ mothers	N	29	19	17	16

Note *Significant difference from group I and group III ($P < 0.05$)
#Geometric Mean Titre

bination of vaccine plus HBIG given at birth which resulted in infection rates between 3 to 15% and a protective efficacy rate in these high risk newborns of 90-100%.[8,11,12,16] When used alone, plasma derived vaccines have decreased the infection rate to 7-30% and a protective efficacy rate of 57.1%.[7] Recently, hepatitis B vaccines have been developed from genetically engineered yeast cells which express the hepatitis B surface antigen. These vaccines are safe and immunogenic in various target groups including neonates.[14,15,19,20] Among 83 infants of HBsAg/HBeAg positive mothers who received hepatitis B immune globulin at birth and three 5 μg doses of yeast-recombinant hepatitis B vaccine, only 4.8% became chronic carriers with 94.6% protective efficacy, similar to that seen with immune globulin and plasma-derived hepatitis B vaccine.[16] Our previous study also showed that a half dose (5 μg) of the recombinant hepatitis B vaccine could be used as effectively as the half standard dose (10 μg) of plasma derived vaccine in the prevention of perinatal HBV transmission in high risk neonates born from HBeAg positive/HBsAg carrier mothers.[12] There is no doubt that combined hepatitis B immune globulin and hepatitis B vaccine give excellent results in the prevention of HBV transmission from high risk carrier mothers to their infants. However, this regimen poses an important economic question for countries where HBIG is scarce and relatively expensive. 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.[15] In the present study we further reduced the dose of recombinant hepatitis B vaccine to 2.5 μg given alone in the same schedule and found that in the high risk infants (group III) who were born from HBsAg/HBeAg positive mothers, the protective efficacy was only 65.7%, which is significantly lower than that obtained using full dose vaccine.[15] However, infants in group I and group II had no evidence of HBV infection up to 13 months of age. The observation that the majority of vaccinated infants up to 95.7-100% had seroconversion after 3 doses of vaccine (at month 4) with relatively high titers of anti-HBs antibody indicated that the low dose (2.5 μg) of the recombinant hepatitis B vaccine is sufficient to produce adequate antibody response in the neonates. After a booster dose of vaccine at 12 months of age, all infants had high anti-HBs antibody responses similar to those obtained from vaccination with full dose vaccine. Although low dose (2.5 μg) of the recombinant HBV vaccine in this study has been shown to be immunogenic and produced adequate antibody response in vaccinated infants, it is less effective in preventing perinatal HBV transmission in high risk infants in group III when compared to the full dose vaccine or the combination of HBIG and low dose vaccine. The effectiveness of the reduced dose of vaccine in the prevention of vertical transmission of HBV has been investigated lately in clinical trials, to lower the total cost of neonatal vaccination.[11,18,21] When our results in group III are compared with those obtained using three low doses (2.5 μg) of plasma-derived vaccine plus HBIG in similar high risk infants, it was found that when three low doses (2.5 μg) of recombinant vaccine alone were used (group III), the seroconversion at 12 months of age was 88.2% and

in infants with positive HBsAg it was 29.2%, while in those receiving HBIG at birth and three low doses (2.5 μg) of plasma-derived vaccine the seroconversion was 74% and in infants with positive HBsAg it was 22%.[18] However, our recent study using the combination of HBIG and four low doses (2 μg) of plasma-derived vaccine revealed that the seroconversion was 86.7% and in infants with positive HBsAg it was 6.7% which was as effective as using the standard dose (5 μg) in the prevention of perinatal HBV transmission in high risk neonates.[11] This study indicated that the low dose (2.5 μg) recombinant hepatitis B vaccine used alone is effective in prevention of vertical HBV transmission in low risk infants (group I and group II), but it is less effective in high risk infants (group III). The low dose hepatitis B vaccine regimen should be combined with HBIG given at birth for the full protection of vertical transmission of HBV infection in the high risk neonates born from HBsAg/HBeAg positive mothers. In an endemic area such as Thailand, this high risk group of infants comprises approximately 3% of all newborns who will require the combination of HBIG and low dose vaccine but the majority of the newborns need only low dose vaccine alone. This approach would certainly reduce the cost of hepatitis B immunization to prevent perinatal HBV transmission and it could be applied economically for the national program of HBV transmission control. Long term follow up of the efficacy of this immunization regimen is in progress.

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