Immunogenicity of two different dosages (10 and 5 μg) of recombinant DNA hepatitis B vaccine in healthy neonates

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The immunogenicity of a half (5 μg) and a full (10 μg) dosage of recombinant DNA yeast-derived hepatitis B vaccine (HB-Vax-DNA) in healthy neonates was assessed in order to compare two candidate dosages of vaccine. After randomization 174 newborns of HBsAg-negative mothers entered the study. Neonates received four doses of either 10 or 5 μg hepatitis B vaccine, according to the DTP–polio immunization schedule at months 3, 4, 5 and 11. No serious adverse reactions were observed; 15.5% of vaccinated newborns suffered mild transient local symptoms. The vaccine was highly immunogenic irrespective of dosage of vaccine; all infants developed anti-HBs levels $\geqslant 10\ IU\ l^{-1}$, *99%* $\geqslant 100\ IU\ l^{-1}$. *A dosage of 10 μg hepatitis B vaccine produced higher antibody levels than 5 μg hepatitis B vaccine after primary vaccination (first three doses) but not after booster vaccination (fourth dose) (*$p = 0.06$ *and 0.75, respectively). Either vaccine dosage can be recommended for incorporation in the Expanded Programme on Immunization in the Netherlands.*

Keywords: Hepatitis B vaccine; immunogenicity; neonates

The WHO strategy for the control of hepatitis B virus (HBV) infection and its sequelae, chronic hepatitis, cirrhosis and hepatocellular carcinoma, is mass vaccination of infants within the framework of the WHO Expanded Programme on Immunization (EPI)[1]. In 1982 plasma-derived hepatitis B vaccine was licensed in The Netherlands and a vaccination programme to determine an effective and practical immunization schedule for the prevention of hepatitis B in neonates of HBsAg positive mothers was initiated[2]. More than 95% of infants developed protective levels of anti-HBs after passive–active immunization[2]. The most effective and immunogenic schedule was achieved with four adult doses of vaccine (10 μg HB-Vax-DNA, Merck Sharp & Dohme (MSD), Westpoint, USA), starting at 3 months of age concomitant with DTP–polio immunization[2,3]. Higher antibody levels were obtained after administration of three adult doses of 10 μg HB-Vax-DNA (GMT anti-HBs at month 12: $1236\ IU\ l^{-1}$) than after three infant doses of 5 μg HB-Vax-DNA (GMT anti-HBs at month 12: $510\ IU\ l^{-1}$), both starting directly after birth[3]. Although an anti-HBs level $\geqslant 10\ IU\ l^{-1}$ is assumed to be protective against HBV[4], an adequate response to a course of vaccine is generally considered to be an anti-HBs level $\geqslant 100\ IU\ l^{-1}$ which will result in longer lasting immunity[5]. HB-Vax-DNA recombinant hepatitis B vaccine was licensed in the Netherlands in 1987. Until now, no studies on the administration of adult and infant doses of HB-Vax-DNA recombinant hepatitis B vaccine, administered at months 3, 4, 5 and 11 to neonates of HBsAg-negative mothers, have been performed.

METHODS

Subjects and randomization

All pregnant women attending the prenatal clinic of the University Hospital Dijkzigt, Rotterdam, the Netherlands, were screened at their first visit for the presence of HBsAg, in addition to the routine determination of blood group/rhesus factor and screening for syphilis. HBsAg-negative mothers were informed about the current hepatitis B immunization programme. Informed consent was obtained from the mother for the participation of her infant. Country of birth and age of the mother, gestational age, birthweight and sex of the

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child were registered on the parturition form. To be accepted into the study, newborns had to weigh at least 2000 g at birth and have a 5-minute Apgar score of 7 or higher. In total, 214 newborns were randomized into groups 1 and 2, according to Peto *et al.*[6]. Forty children were not included in the study for the following reasons: secondary refusal of the parents ($n=30$), birthweight less than 2000 g ($n=4$), Apgar score less than 7 ($n=5$), perinatal death ($n=1$). As a result, 86 neonates of group 1 and 88 of group 2 entered the study (intention to treat).

Vaccine and vaccination schemes

The ad antigenic recombinant DNA yeast-derived hepatitis B vaccine was provided by Merck Sharp & Dohme (10 μg and 5 μg HB-Vax-DNA, Westpoint, USA); 10 μg HBsAg were contained in 1.0 ml, 5 μg HBsAg in 0.5 ml, both in single-dose vials (lot numbers 496750P, 557860P, 5806990S, 609540S, 651450S for 10 μg HB-Vax-DNA and 460201N, 460202N, 088011CH-B, 580700S, 623040S,676710T for 5 μg HB-Vax-DNA). The production, quality control and physicochemical characteristics of HB-Vax-DNA have been described elsewhere[7]. The vaccine, stored at 2–8°C, was administered into the quadriceps muscle by a physician. Parents were asked to record any local (soreness, redness, swelling) or systemic (fever >37.5°C, gastrointestinal symptoms) symptoms over 3 days following each injection of vaccine. Concomitant side-effects known to be due to DTP–polio immunization given at the same time were excluded from analysis. The 86 neonates of group 1 received four doses of 10 μg vaccine and 88 neonates of group 2 received four doses of 5 μg vaccine. The vaccine was administered to both groups at months 3, 4, 5 (primary vaccination) and 11 (booster vaccination).

Blood tests and laboratory methods

Two millilitres of blood were drawn at month 0 (umbilical cord blood) and months 4, 6, 11 and 12 in both groups. HBsAg, anti-HBc and anti-HBs were measured by radioimmunoassay (Ausria II, Corab, Ausab, Abbott Laboratories, Chicago, IL, USA). Anti-HBs is expressed in International units/litre (IU l^{-1}) after comparison with the WHO standard preparation (Central Laboratory of the Netherlands Red Cross Blood Transfusion Services, Amsterdam, The Netherlands).

Statistics

Statistical analysis was applied on two datasets: data according to intention-to-treat data and clean data (data of all children who entered the study and were vaccinated and assessed according to the protocol). Results between 'intention to treat data' and 'clean data' did not differ. The results of analysis of the clean data are presented below. Differences in discrete variables were analysed by Fisher's exact test and χ^2 test. Continuous variables were analysed by the two-sample Wilcoxon rank sum test for unpaired observations. The limit for significance was set at 0.05. Geometric mean anti-HBs levels (GMTs) are expressed in IU l^{-1} with 95% confidence intervals (CI).

RESULTS

From April 1990 to March 1992, 174 infants born of HBsAg-negative mothers entered the study and were vaccinated according to the protocol. After primary vaccination serum samples were obtained in 73 (85%) and 79 (90%) cases in groups 1 and 2, respectively. After booster vaccination, 66 (77%) and 77 (88%) samples were available. There were no significant differences in country of birth of the mother, median age of the mother, median gestational age, median birthweight, sex of the child and number of infants with anti-HBs positivity and anti-HBc positivity in cord blood between the two groups (*Table 1*).

Passive transfer of maternal antibodies

Passively acquired anti-HBs positivity rates for cord blood were 13% ($n=11$) and 15% ($n=13$) for groups 1 and 2, respectively. Anti-HBc positivity rates at birth were 12% ($n=10$) and 13% ($n=11$), respectively. In all but one case anti-HBc was negative at month 12 (in one case the test result was not available).

Anti-HBs seroconversion rate

Figure 1 shows the frequencies of protective levels of anti-HBs levels 1 month after primary and booster vaccination in the two groups. All infants who received 10 μg of vaccine (group 1) developed protective anti-HBs levels (≥ 10 IU l^{-1}) after primary vaccination. All infants who received 5 μg of vaccine (group 2) developed anti-HBs levels ≥ 10 IU l^{-1}, but in two cases this did not occur until after the booster vaccination. As far as long-term protection is concerned, 100% of infants in group 1 had anti-HBs levels ≥ 100 IU l^{-1} after the booster vaccination; for group 2 the corresponding percentage was 99%; one child had an anti-HBs level between 10 and 100 IU l^{-1}. Ultimately 88% of the infants in group 1 had anti-HBs levels ≥ 1000 IU l^{-1} after the booster vaccination in comparison to 90% of the infants in group 2.

The anti-HBs levels for two children in group 2 were 1 IU l^{-1} and 6 IU l^{-1}, respectively, after primary vaccination. After the booster vaccination these children exhibited 64 and 8078 IU l^{-1}, respectively.

Table 2 lists the anti-HBs levels (GMT) with 95% CI (anti-HBs >0 IU l^{-1}) for the two different schedules. The GMT anti-HBs, obtained 1 month after primary vaccination and after booster vaccination with four doses of 10 μg HB-Vax-DNA, were 231 IU l^{-1} and 4119 IU l^{-1},

Table 1 Distribution of the entry variables according to vaccination groups 1 and 2

Variable	Group 1	Group 2	*p* value
Number of infants (*n*)	86	88	
Median gestational age (weeks)	40	39	0.20[a]
Median birthweight (g)	3323	3218	0.16[a]
Sex of child (*n*):			0.76[b]
Male	51	50	
Female	35	38	
Anti-HBs positive (*n*)	11	13	0.83[b]
Anti-HBc positive (*n*)	10	11	1.00[b]
Median age of mother (years)	26	29	0.20[a]
Country of birth of mother (*n*):			0.45[c]
Netherlands	28	23	
Mediterranean	12	17	
Surinam	29	25	
Asian	1	4	
Miscellaneous	16	19	

[a]Wilcoxon rank sum test
[b]Fisher's exact test
[c]χ^2 test

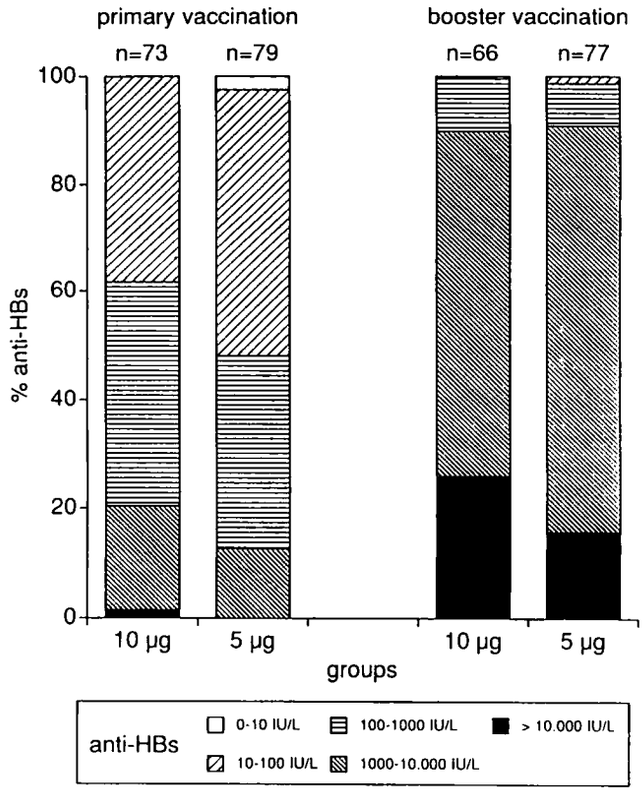

Figure 1 Percentage of infants with various anti-HBs levels after primary and booster vaccination with 10 µg and 5 µg HB-Vax-DNA hepatitis B vaccine administered at 3, 4, 5 and 11 months of age

Table 2 Immunogenicity 1 month after primary and booster vaccinations with 10 µg and 5 µg HB-Vax-DNA hepatitis B vaccine administered at 3, 4, 5 and 11 months

Group	No.	Month	Anti-HBs (>0 IU l^{-1}) (GMT)	95% CI	*p* value
1 month after primary vaccination					
1 (10 µg)	73	6	231	163–328	0.06[a]
2 (5 µg)	79	6	137	99–190	
1 month after booster vaccination					
1 (10 µg)	66	12	4119	3064–5538	0.75[a]
2 (5 µg)	77	12	3823	3008–4859	

[a]Wilcoxon test scheme 1 versus 2

respectively. This tends to be significantly (Wilcoxon test: $p=0.06$) higher than those found for infants in group 2 after primary vaccination (GMT anti-HBs: 137 IU l^{-1}) but not (Wilcoxon test: $p=0.75$) after booster vaccination (GMT anti-HBs: 3823 IU l^{-1}).

Anti-HBs levels at month 24 were available for 32 infants of group 1 (GMT anti-HBs 290 IU l^{-1}, 95% CI: 187–449) and 36 infants of group 2 (GMT anti-HBs 231 IU l^{-1}, 95% CI: 138–386).

No significant differences in GMT anti-HBs were found between boys and girls, presence of passively acquired maternal antibodies (anti-HBs, anti-HBc) and various countries of birth of the mother (results not shown).

Adverse reactions to vaccine

No cases of clinical hepatitis or serious side-effects of the vaccine were reported. In 27 cases (15.5%) the parents of the vaccinated children reported transient red swelling at the site of injection or fever (less than 39°C). No significant differences in the number of side-effects between infants in group 1 and group 2 were found (results not shown).

DISCUSSION

The 10 µg and 5 µg HB-Vax-DNA hepatitis B vaccines used in this study were safe and highly immunogenic. No serious side-effects were reported. In 15.5% of the infants mild transient local symptoms were present, irrespective of the dose of vaccine. This finding is in agreement with earlier results[8,9].

All infants reached anti-HBs levels ≥ 10 IU l^{-1}, 99% ≥ 100 IU l^{-1}, after completion of the vaccination schedule (*Figure 1*). In *Table 3* the results of different studies on the immunogenicity of at least three doses of recombinant hepatitis B vaccine (MSD) in healthy neonates and infants are listed. Although there is agreement in the seroconversion rates, the GMT anti-HBs differs (median 1694 IU l^{-1}, range 307–4119 IU l^{-1}). These differences might be explained by differences in maternal HBsAg status, vaccine, dosage, schedule, time of blood sampling, age and ethnic background of the vaccinees.

In comparison with a full dosage (20 µg) of Engerix-B recombinant hepatitis B vaccine (SmithKline Beecham Biologicals (SKB)) administered at months 3, 4, 5 and 11 to neonates of HBsAg-negative mothers[10], GMT

Table 3 Immunogenicity of at least three doses of hepatitis B recombinant vaccine (HB-Vax-DNA, Merck Sharp & Dohme) in healthy neonates and infants

Author (year of publication)	Dose (µg HBsAg)	Scheme in months	No. of vaccinees	Age-group (neonate's mother HBsAg +/−)	Seroconversion rate (%)[a]	GMT anti-HBs of converters (95% CI)
Lai (1986)[19]	5	0, 1 and 6	21	Infants Neonates (+)	100 (2)	1894 (?)
Stevens (1987)[13]	5	0, 1 and 6	83	Neonates (+)	100 (6)	307 (?)
Milne (1988)[9]	10	0, 1 and 6	56	Infants	98 (2)	3246 (2272–4636)
	5	0, 1 and 6	60		98 (2)	2305 (1638–3158)
	2.5	0, 1 and 6	56		98 (2)	1370 (866–2169)
Stevens (1990)[20]	5	0, 1, 2 and 6	94	Neonates (+)	96 (3)	509 (?)
Goh (1992)[18]	5	0, 1 and 6	31	Infants	100 (3)	1699 (?)
	2.5	0, 1 and 6	30		93 (3)	1689 (?)
	1.25	0, 1 and 6	29		100 (3)	1135 (?)
	0.6	0, 1 and 6	31		95 (3)	1088 (?)
Canho del (present study)	10	3, 4, 5 and 11	65	Neonates (−)	100 (1)	4119 (3064–5538)
	5	3, 4, 5 and 11	71	Neonates (−)	100 (1)	3823 (3008–4859)

[a]Months after completion of vaccination scheme are given in parentheses

anti-HBs at months 12 and 24 were 20 768 IU l^{-1} and 2957 IU l^{-1}, respectively, five and ten times higher than those obtained with 10 μg HB-Vax-DNA in the present study. The seroconversion rate for 20 μg Engerix-B recombinant vaccine (SKB) was the same as that for 10 μg HB-Vax-DNA recombinant vaccine (MSD), both being 100%[10]. In another study four doses of 10 μg Engerix-B resulted in GMT anti-HBs of 2565 IU l^{-1} 1 month after the fourth dose, which is 1.5 times lower than in the present study; the seroconversion rate was 97.1%[11].

Our previous study[2] with 10 μg plasma vaccine (MSD), administered at months 3, 4, 5 and 11, showed that plasma vaccine gives a significantly higher (Wilcoxon rank-sum test $p=0.0001$) anti-HBs response ($n=99$, peak level at month 12: 13 427 IU l^{-1}) than 5 and 10 μg of the recombinant vaccine evaluated in the present study ($n=143$, peak level groups 1 and 2 at month 12: 3957 IU l^{-1}). This finding is in agreement with previous studies[12,13]. The proportional decline in anti-HBs between 12 and 24 months was not significantly different (Wilcoxon rank-sum test $p=0.3572$) between plasma vaccine (91%, 95% CI: 21–98) and recombinant vaccine (91%, 95% CI: 71–98). This is in agreement with the study of Jilg *et al.*[14], who found a 90% reduction in anti-HBs, 12 months after the last vaccination with 20 μg and 5 μg plasma vaccine. However, more long-term immunogenicity studies on recombinant hepatitis B vaccine are needed to determine whether the decline of antibodies resembles that for plasma hepatitis B vaccine.

The recommended dosage of vaccine for neonates is ambiguous; Dutch authorities advise a full dosage of 10 μg HB-Vax-DNA[15]; the US Immunization Advisory Committee recommends half this standard adult dosage, 5 μg[16].

Recently, 2 μg and 2.5 μg doses of HB-Vax-DNA were recommended for the national childhood immunization programme because no significant differences in seroconversion rates could be found between 2.5, 5 and 10 μg HB-Vax-DNA, administered at months 0, 1 and 6 to anti-HBs negative infants, after completion of the vaccination schedule[18]. Much lower dosages of 0.6 μg and 1.25 μg HB-Vax-DNA were even as immunogenic as 2.5 μg, but not before month 9[18]. We found that 5 μg HB-Vax-DNA builds up a lower anti-HBs level than 10 μg HB-Vax-DNA after primary vaccination but that difference disappears after the booster vaccination.

The conclusion is that lower dosages of vaccine require more time to build up the same immunogenicity. For universal vaccination of infants of HBsAg-negative mothers the rapidity of acquiring the required immunogenicity is less important than for infants of HBsAg-positive mothers, who are at greatest risk for becoming chronic carriers of HBV. It appears that health authorities must opt for that vaccine dosage that will satisfy the task they set up.

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