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Immunogenicity of a Low Dose Recombinant DNA Hepatitis B Vaccine in Healthy Adults in Singapore

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SUMMARY **The immunogenicity and safety of a standard dose of 10 μg of a yeast derived recombinant DNA hepatitis B vaccine (B-Hepavac II) was compared with that of a reduced dose of 5 μg in 84 healthy adult volunteers with no previous exposure to hepatitis B. Each subject received either a 10 μg or 5 μg dose of vaccine intramuscularly at 0, 1 and 6 months. One month after the second injection of vaccine the seroconversion rate in the two groups were 85 and 86 percent respectively. Two months after the third injection 100 percent of participants had seroconverted; 95 percent of the 10 μg group and 91 percent of the 5 μg group had titres of anti-HBs greater than 10 IU/L. The geometric mean titres (GMT) of anti-HBs levels at 2, 6, 8, and 12 months were 34, 61, 811 and 188 IU/L in the 10 μg group and 26, 45, 836 and 304 IU/L in the 5 μg group respectively. Adverse effects were mild and transient. The vaccine was safe and immunogenic in the doses given. The reduced dose of 5 μg was as effective as the standard 10 μg dose.**

Plasma derived hepatitis B vaccine has been in use in Singapore for the past 10 years and has been found to be safe, immunogenic and effective in preventing hepatitis B virus (HBV) infection. Studies have shown that half the recommended dose of the plasma vaccine is as effective in inducing an immunological response as the full dose.[1] Lately, at least two recombinant yeast derived hepatitis B vaccine have been introduced (Merck Sharpe & Dohme : B-Hepavac II, Smith Kline Biologicals: Engerix B). The recommended dose of 10 μg of the MSD B-Hepavac II has been found to induce an immunologic response in up to 100 percent of healthy adults.[2-4] In a local evaluation recently, using a similar dose, the response rate in healthy hospital adults was 100 percent, 97 percent of them having antibody levels above 10 IU/L.[2]

The present study compares the immunogenicity of a reduced dose of the recombinant hepatitis B vaccine with that of the full recommended dose in healthy adult volunteers in Singapore.

MATERIALS AND METHODS

Subjects

Healthy adult volunteers who were negative for serological markers of HBV infection, i.e. hepatitis B surface antigen (HBsAg), antibody to hepatitis B surface antigen (anti-HBs) and antibody to hepatitis B core antigen (anti-HBc) were included in the study. Subjects with intercurrent infections, pregnancy, diabetes mellitus or renal failure were excluded from the study. Informed consent was obtained from all volunteers.

Vaccine

Merck Sharp and Dohme yeast derived recombinant DNA (MSD rDNA) hepatitis B vaccine (Lot No. E6720) was given to the two groups of volunteers. Each 1 ml dose of the vaccine contained 10 μg hepatitis B surface antigen adsorbed onto 0.5 mg aluminium hydroxide with thimerosol 1:20,000 added as a preservative. The vaccine was stored at 2° to 8° C prior to use and was given into the upper deltoid muscle in all subjects.

Study design

The study was carried out over a 15 month period. The subjects were randomised into two groups receiving either three doses of 5 μg or three doses of 10 μg MSD rDNA

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Hepatitis B vaccine. All doses were given at time, 0, 1 and 6 months. A booster dose of 10 μg of the same vaccine was given at month 14 for volunteers with anti-HBs levels below 10 IU/L at 12 months. These subjects were observed for another month to determine antibody response to the booster dose.

Subjects were taught to use mercury thermometers to record their temperatures daily for five days after each vaccination. Local or systemic adverse effects were recorded in diary cards.

Blood tests

Blood samples were obtained at -2 weeks, 0, 1, 2, 4, 6, 8 and 12 months after the first vaccination dose. These were analysed for serum alanine aminotransferase (ALT) activity, HBsAg, Anti-HBs and Anti-HBc. Additional blood samples were obtained at 14, 14½ and 15 months from subjects receiving the booster dose of vaccine at month 14 and analysed for anti-HBs.

Laboratory methods

Serum alanine aminotransferase activity (ALT) was assessed by a Greiner analyser G-400 (Greiner Electronics Diagnostica, Switzerland). The normal value was < 40 IU/L. Anti-HBc was measured by enzyme immunoassay (EIA) (CORZYME Abbott Laboratories, North Chicago, Illinois). HBsAg was measured by EIA (AUSZYME Poly, Abbott Laboratories, North Chicago, Illinois). Anti-HBs was determined by using radioimmunoassay (AUSAB RIA; Abbott Laboratories, North Chicago, Illinois). Anti-HBs titres based on the RIA procedure was calculated in international units/litre (IU/L) based on a WHO reference standard. Seroconversion is defined as a rise in Anti-HBs titre to $\geqslant 2.1$ IU/L as titres less than this has been found to be unreliable.

Statistical analysis

Anti-HBs development (seroconversion rates) at months 1, 2, 4, 6, 8 and 12 were compared in the two groups of subjects using the chi-square test with Yate's correction. Comparisons of GMT between anti-HBs positive patients ($\geqslant 10$ IU/L) at months 1, 2, 4, 6, 8 and 12 were performed using the non-parametric Mann-Whitney tests. All P values less than 0.05 (two-tailed) were considered significant.

RESULTS

Eighty Four volunteers were recruited into the study: 43 of them received 5 μg per dose and 41 received 10 μg per dose. Characteristics of these volunteers are detailed in Table 1.

Anti-HBs response to vaccine

A comparison of the anti-HBs response between the two groups is shown in Table 2 and 3. One month after the first two injections of vaccine the seroconversion rate (defined as anti-HBs $\geqslant 2.1$ IU/L) in the 10 μg and 5 μg groups were 85 and 86 percent respectively. Two months after the third injection 100 percent of the participants had seroconverted. Ninety-five percent of the 10 μg group and 91 percent of the 5 μg group had titres of anti-HBs greater than 10 IU/L (Table 2). The differences were not statistically significant.

There was no significant difference between the anti-HBs geometric mean titres (GMT) of seroconverters with anti-HBs $\geqslant 10$ IU/L in both groups at each of the assessment period (Table 3). Although a majority of the volunteers in both groups seroconverted six months after the first vaccine dose (Table 2), the anti-HBs GMT among seroconverters with anti-HBs $\geqslant 10$ IU/L remained relatively low, 61 IU/L and 45 IU/L in the 10 μg and 5 μg groups respectively. The anti-HBs GMT in both groups rose sharply 2 months after the third vaccine dose. The rise in GMT in seroconverters with anti-HBs $\geqslant 10$ IU/L from month 6 to month 8 was statistically signi-

Table 1. Details of subjects

	Vaccine Dose	
	5 μg	10 μg
Number of subjects	43	41
Sex :—Male : Female	18 : 25	15 : 26
Mean Age (Range)	27 (15–43)	32 (16–56)
Race :—Chinese : Indian : Malay : Eurasian	41 : 1 : 0 : 1	36 : 1 : 3 : 1

Table 2. Anti–HBs response by dose in adult volunteers given 3 doses of MSD yeast–derived recombinant hepatitis B vaccine at time 0, 1 and 6 months

	No. of subjects developing Anti–HBs (%)			
	Anti–HBs $\geqslant 2.1$ IU/L		Anti–HBs $\geqslant 10.0$ IU/L	
Months	10 μg	5 μg	10 μg	5 μg
1	18/41 (44)	11/43 (26)	4/41 (10)	2/43 (5)
2	34/40 (85)	37/43 (86)	20/40 (50)	17/43 (40)
4	38/41 (93)	37/43 (86)	33/41 (81)	24/43 (56)
6	37/41 (90)	37/43 (86)	33/41 (81)	24/43 (56)
8	41/41 (100)	43/43 (100)	39/41 (95)	39/43 (91)
12	39/40 (98)	42/43 (98)	38/40 (95)	38/43 (88)

ficant in both the 10 μg and 5 μg groups ($p < 0.0001$).

"Low-responders"

Although sero-conversion rates were 100 percent in both groups, six subjects did not have protective levels of anti-HBs (defined as anti-HBs ⩾ 10 IU/L) two months after the third vaccine dose. Two of them were in the 10 μg group. All except one subject in the 5 μg group were aged 30 and above. At month 12 anti-HBs fell to below protective level in one female responder (TOL) in the 5 μg group and to undetectable levels in one of the low responders in the 10 μg group. Antibody levels were measured in all these low responders at month 14 and were found to be undetectable in both subjects in the 10 μg group and in two of five subjects in the 5 μg group. All seven subjects responded after a booster dose of 10 μg of the MSD rDNA hepatitis B vaccine given at month 14. One subject, however, failed to achieve an anti-HBs level of 10 IU/L (Table 4).

Side effects and hepatitis events

No serious adverse effects were observed after vaccination. After the 1st vaccine dose, 10 volunteers complained of soreness at the injection site while fever was observed in 7 of them. Other minor side effects included giddiness and malaise which improved spontaneously, and a transient skin rash after the first dose in one person. Side effects generally disappeared with subsequent doses. During the 12 months of observation, none of the volunteers became positive for HBsAg or anti-HBc.

Eight subjects had elevated ALT from sera drawn on the day of the 1st vaccine dose. Four had values less than 50 IU/L, 2 had values between 50 and 100 IU/L, 2 others had levels of 103 and 122 IU/L respectively. Subsequent results were normal. Two subjects were found to have viral infections (sorethroat

Table 3. Geometric mean anti–HBs titres of sero-converters (Anti–HBs ⩾ 10 IU/L) in adult volunteers given 3 doses of MSD recombinant yeast–derived hepatitis B vaccine at time 0, 1 and 6 months

Months	GMT (IU/L) (1 SD range) 10 μg	5 μg
1	66 (13–337)	26 (18–37)
2	34 (12–95)	26 (13–47)
4	60 (20–176)	44 (19–101)
6	61[a] (23–162)	45[d] (17–116)
8	811[b] (182–3,610)	836[e] (118–5,910)
12	188[c] (47–753)	304[f] (50–1,827)

a vs b : $p < 0.0001$, b vs e : $p < 0.0001$,
a vs d, b vs e, c vs f : Not significant

Table 4. Anti–HBs titres of "Low Responders" (Anti–HBs < 10 IU/L at month 12) in adult volunteers given 3 doses of MSD recombinant yeast–derived hepatitis B vaccine at months 0, 1 and 6. Booster dose of the vaccine (10μg) was given at month 14

Patient	Sex/Age	Vaccine Dose	Anti–HBs titres (IU/L) at months 8	12	14	14½	15
WC	M/38	10 μg	2.3	0	0	36.1	23.2
WOR	F/56	10 μg	3.5	3.7	0	2.4	4.3
SSH	M/20	5 μg	8.0	4.8	4.3	242.0	92.9
TOL	F/27	5 μg	36.9	5.5	2.7	952.0	2951.0
WTT	M/43	5 μg	6.5	2.8	0	20.9	40.5
LDP	F/30	5 μg	9.8	6.5	3.3	61.9	272.0
WKS	M/30	5 μg	3.1	1.9	0	14.5	54.2

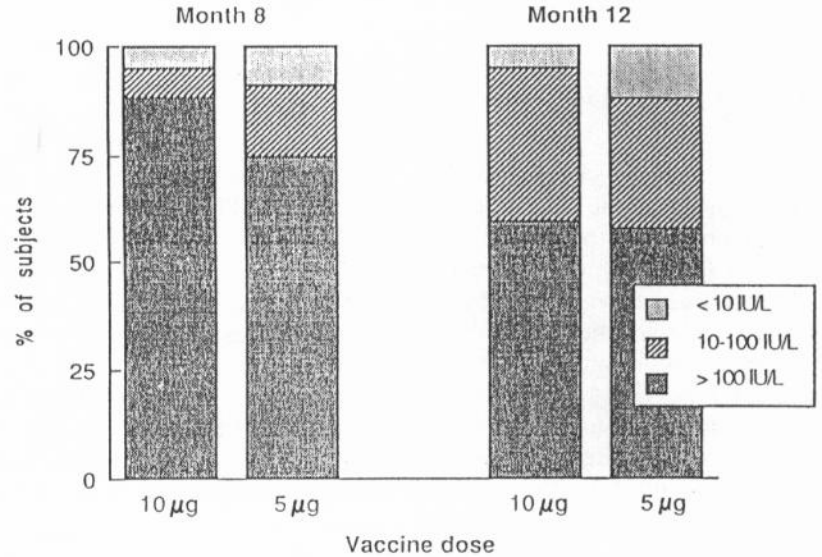

Fig. 1 Anti–HBs levels at month 8 and 12

and flu). No explanation for these elevations were available on history and clinical examination of the other subjects.

DISCUSSION

Viral Hepatitis B is endemic in Singapore and the carrier rate is about 5 percent.[1] Infection during childhood forms the main bulk of the carrier pool. Vaccination is the only effective means of preventing hepatitis B infection and its sequelae of chronic liver disease and hepatocellular carcinoma. The cost of recombinant DNA (rDNA) hepatitis B vaccine varies in different counties. Until recently, a course of three injections of 10 μg of B-Hepavac II would cost around S$180.00 (US$ 90). The recommended dose of 10 μg for adults has been found to give a seroconversion rate of 100 percent in healthy hospital staff recently.[2] Previous studies have shown that with the plasma vaccine, half the recommended dose was as effective in inducing an immunological response as the full dose.[1] In this study, we have demonstrated good immunogenicity using half the recommended dose of the recombinant vaccine. Anti-HBs developed rapidly, with 85 percent of the 10 μg group and 86 percent of the 5 μg group becoming positive one month after the second dose (Table 2). This is in agreement with studies done elsewhere.[3,4]

The higher levels of anti-HBs in the 5 μg group at months 8 and 12 when compared to that in the 10 μg group (although not statistically significant) can be explained by the lower mean age of these individuals. Among hyporesponders and one "secondary vaccine failure", anamnestic immune response was observed when a booster dose of 10 μg was given (at month 14). Six of them responded with levels ranging from 23 to 2951 IU/L (Table 4), one month after the booster dose. The antibody response after the booster injection was much better in subjects who had 5 μg doses than that observed in subjects in the 10 μg group. Those low responders in the 10 μg group can therefore be considered true hyporesponders.

About 60 percent of the subjects in both dosage groups had antibody levels greater than 100 IU/L at month 12 (Fig. 1). In these subjects, a booster dose will probably not be required until 3 to 4 years later.[5] There is evidence that even when antibody level against HBsAg become undetectable, a vaccinated individual seems to remain protected against clinically significant diseases if the initial antibody response was above the protective level.[6] The incubation period of hepatitis B usually exceeds 6 weeks. It is likely that exposure to hepatitis B virus will induce an anti-HBs response during the early phase of the incubation period thereby preventing or modifying an infection. The volunteers in this study are being followed at yearly intervals to determine the natural history of antibody levels of vaccinated subjects.

In the treatment or prevention of any disease the minimum effective dose should obviously be used. The fact that the 5 μg dose is as effective as the 10 μg dose in inducing an immune response in healthy adults has tremendous economic implications and could possibly bring HB vaccination within reach of the whole population. The Singapore Government is encouraging this form of prophylaxis by allowing part payment through the medisave programe. With the recent drop in price of the vaccine and the introduction of a new lot with higher immunogenicity, there should be no reason why Singapore will not be hepatitis B free in the foreseeable future. It also would make control of hepatitis B an economically realistic option in many countries where the disease is endemic.

In summary, the yeast recombinant DNA hepatitis B vaccine was safe and immunogenic in the doses given. The new lot of MSD B-Hepavac II would ensure increased anti-HBs levels. It is prudent, however, to measure anti-HBs levels after the 3rd vaccination dose especially if the 5 μg dose is given. Low responders to this low dose regime can then be identified and given a single booster vaccination at the full recommended dose.

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