Long-term immunogenicity and efficacy of a reduced dose of plasma-based hepatitis B vaccine in young adults

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A cohort of seronegative preclinical medical and dental students and another cohort of seronegative national service recruits who were immunized intramuscularly with a reduced dose (10 μg) of plasma-based hepatitis B vaccine (Merck, Sharp & Dohme) at the start of the study and at 1 month and 6 months thereafter were followed up for 5 years and 6 years, respectively. Among the medical and dental students, antibody to hepatitis B surface antigen (anti-HBs) (≥10 mIU/ml) was detected in 81% of the vaccinees at the end of the 5-year follow-up and the geometric mean titre (GMT) had dropped from 412.6 mIU/ml one year after completion of vaccination to 174.9 mIU/ml after 5 years. Antibody to hepatitis B core antigen (anti-HBc) was detected in 0.4–1.0% of the vaccinees but none was positive for hepatitis B surface antigen (HBsAg) during the follow-up period.

Among the national servicemen, the anti-HBs seroconversion rate and GMT were considerably lower than those of the preclinical medical and dental students. At the end of the 6-year follow-up, 55% of the vaccinees were positive for anti-HBs (≥10 mIU/ml) and the GMT had dropped from 80.7 mIU/ml one year after completion of vaccination to 30.4 mIU/ml after 6 years. Anti-HBc was detected in 8 (2.7%) and transient HBs antigenaemia in 2 (0.7%) of 293 vaccinees after 4 years. However, none of the 196 vaccinees followed up at 6 years was HBsAg-positive compared with 8 (4.2%) of 191 seronegative national service recruits who were not vaccinated, giving a vaccine efficacy of 100% in the prevention of the chronic HBsAg carrier state.

Introduction

When hepatitis B vaccine first became commercially available, it was extremely expensive. Clinical trials on the immunogenicity of reduced doses of the vaccine were therefore conducted in several centres. These studies showed that the dose of vaccines from some manufacturers could be reduced without affecting the vaccine-induced antibody response in newborns, infants and adults (*1–7*). A reduced dose of 5 μg of Merck, Sharp & Dohme (MSD) plasma-based vaccine is as immunogenic and efficacious as the standard dose of 10 μg in preventing perinatal transmission of hepatitis B virus (HBV) (*8*). In healthy adults immunized with the MSD plasma-based vaccine, no significant difference in the immune response was observed between groups receiving doses of 40 μg, 20 μg, and 10 μg, and there was 92% seroconversion with the 5-μg dose (*9–12*). Similarly, clinical trials with MSD yeast-derived HBV vaccine demonstrated that, for pre-exposure prophylaxis in children aged 1–12 years, the immunogenicity of lower doses (0.6 μg, 1.25 μg, and 2.5 μg) was as good as that of the recommended dose (5 μg) (*13*). The immunogenicity of reduced doses was also demonstrated for teenagers (2.5 μg) (*14–15*) and adults (*16*) (5.0 μg). These findings are of practical importance, especially in developing countries where hepatitis B virus infection is endemic, since the use of a lower dose of vaccine without compromising its immunogenicity and efficacy would reduce considerably the cost of immunization programmes.

In the study, we followed up for 5–6 years two cohorts of seronegative adults who were immunized with a reduced dose of 10 μg of MSD plasma-based HBV vaccine. To the best of our knowledge this is the first long-term follow-up of vaccinees administered such a dose.

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Materials and methods

The population groups followed up consisted of preclinical medical and dental students and national service recruits. The Singapore Expert Committee on the Immunisation Programme has recommended that these groups should be protected against viral hepatitis B on a voluntary basis (*17*).

The purpose of the study was carefully explained to the volunteers and their informed consent was obtained. Prior to vaccination, 5 ml of venous blood was collected from each subject, using disposable needles and syringes, for analyses of various HBV markers. Blood collection was carried out at the Student Health Service Clinic, National University of Singapore, for the students, and at the medical centre of one of the army camps, for the national service recruits.

The blood samples were immediately dispatched to the Hepatitis and Liver Cancer Research Unit, Department of Clinical Research, Singapore General Hospital. The sera were separated, transferred to labelled polypropylene tubes, and stored at –70 °C before being analysed in batches. The samples were tested for hepatitis B surface antigen (HBsAg), antibody to hepatitis B core antigen (anti-HBc), and antibody to hepatitis B surface antigen (anti-HBs) using commercially available enzyme immunoassay kits (AUZYME II, CORZYME and AUSAB, resp.).[a]

Subjects who were negative for HBsAg, anti-HBc and anti-HBs and who had no previous history of hepatitis B vaccination were offered three 10-µg doses of MSD plasma-based HBV vaccine, administered intramuscularly in the deltoid region, at the start of the study and 1 month and 6 months later. Blood samples were obtained 1, 2, 3, and 5 years after completion of the immunization schedule from the students and after 1, 2, 4, and 6 years from the national service recruits. Since it would not have been ethical to obtain periodic blood samples from those who refused immunization, we tested the sera of unvaccinated national service recruits for HBV markers only once at the end of the 6-year follow-up period. Sera were tested for HBsAg, anti-HBc and anti-HBs. The reciprocal anti-HBs titres were expressed in mIU/ml, based on a WHO reference standard.[b] Seroconversion was defined as an increase in anti-HBs titre to ≥2.1 mIU/ml. The difference in HBV markers between vaccinated and unvaccinated groups was examined using Fisher's exact test; a *P*-value of <0.05 was considered to be statistically significant. The efficacy of the vaccine (*VE*) in preventing HBV infection at the end of the follow-up period was calculated using the following equation:

$$VE\ (\%) = \frac{U - V}{U} \times 100$$

where U = % of unvaccinated individuals positive for HBV markers

and V = % of vaccinees positive for HBV markers.

Results

A total of 240 seronegative preclinical medical and dental students aged 19–21 years and 293 seronegative national service recruits aged 18–21 years were immunized and followed up for 5 years and 6 years, respectively. At the end of the 6-year follow-up period, 191 unvaccinated national service recruits were also tested for various HBV markers. All the vaccinees remained well and no clinical hepatitis was reported during the follow-up period.

Preclinical medical and dental students

One year after completion of vaccination, 98.3% of the students had seroconverted, 93.6% having anti-HBs titres ≥10 mIU/ml and 32.3% ≥1000 mIU/ml. The geometric mean titre (GMT) dropped from 412.6 mIU/ml after 1 year to 133.6 mIU/ml after 3 years, and then increased again to 174.9 mIU/ml after 5 years. At the end of the follow-up period, more than 80% of the students continued to have protective levels of anti-HBs (≥10 mIU/ml) with about 60% having levels ≥100 mIU/ml (Table 1). Anti-HBc, a marker of HBV infection, was detected

Table 1: **Anti-HBs titres of medical and dental students immunized with three 10-µg doses of MSD plasma-based hepatitis B vaccine and followed up for 5 years[a]**

Anti-HBs titre (mIU/ml)	% at post-vaccination follow-up after:			
	1 year (*n* = 240)	2 years (*n* = 219)	3 years (*n* = 191)	5 years (*n* = 100)
0	2.2	5.9	2.6	7
<10	4.2	9.6	11.0	12
10 to <100	20.0	33.8	28.8	22
100 to <1000	41.3	31.1	42.4	40
≥1000	32.3	19.6	15.2	19
Geometric mean titre	412.6	145.8	133.6	174.9

[a] Each vaccinee received 1 dose of vaccine at the beginning of the study and 1 month and 6 months later.

[a] Abbott Laboratories, North Chicago, IL, USA.

[b] Supplied by: International Laboratory for Biological Standards, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, Netherlands.

in 0.4–1.0% of the vaccinees, but none was positive for HBsAg (Table 2).

National service recruits

For the national service recruits the seroconversion rate 1 year after completion of vaccination was 70.4%; after 2 years, 83.1%; after 4 years, 79.6%; and after 6 years, 76.3%. The GMT dropped from 80.7 mIU/ml after 1 year to 30.4 mIU/ml after 6 years. More than half (55%) of the vaccinees continued to have anti-HBs levels ≥10 mIU/ml at the end of the follow-up period, with 22.5% having levels ≥100 mIU/ml (Table 3).

The prevalence of anti-HBc increased from 0.9% after 1 year to 2.7% after 4 years and 2.0% after 6 years. HBsAg was weakly positive in two vaccinees after 4 years, but disappeared completely on repeat testing of subsequent samples.

When a cohort of 191 unvaccinated seronegative national service recruits from the same army camp was tested for HBV markers at the 6-year follow-up, 8 (4.2%) were HBsAg-positive, 40 (20.9%) were anti-HBc-positive, and 57 (29.8%) were anti-HBs-positive (Table 4). The difference in the prevalence of HBsAg and anti-HBc between the vaccinated and unvaccinated national servicemen at the 6-year follow-up was statistically significant (P <0.01 and P <10^{-8}, resp.), and the vaccine efficacy in preventing the HBsAg carrier state and HBV infection (anti-HBc positivity) in vaccinees was 100% and 90.4%, respectively.

Discussion

The study was based on the follow-up of two cohorts of seronegative young adults immunized in a normal setting rather than under strict clinical trial conditions. The dose of vaccine administered to young national servicemen was highly effective in preventing HBV infection (as indicated by anti-HBc positivity) and the HBsAg carrier state. Although there was no control group to assess the efficacy of the vaccine among the medical and dental students, the protection appeared to be equally good. In Singapore dental surgeons have a significantly higher prevalence of HBsAg and anti-HBc (11.4% and 45.6%, resp.) than that of the general population (4.2% and 29.7%, resp.) (*18*).

The immune response of the medical and dental students was better than that of the national servicemen, with their GMT being 3–5 times higher after 1–2 years' follow-up. This difference could be due to variations in immunogenicity between individual

Table 2: **Hepatitis B virus markers in medical and dental students immunized with three 10-µg doses of MSD plasma-based hepatitis B vaccine and followed up for 5 years**[a]

Marker[b]	% +ve at post-vaccination follow-up after: 1 year (n = 240)	2 years (n = 219)	3 years (n = 191)	5 years (n = 100)
HBsAg	0	0	0	0
Anti-HBc	0.4	0.5	0.5	1.0
Anti-HBs	98.3	94.1	97.4	93.0

[a] See footnote *a*, Table 1.
[b] HBsAg = hepatitis B surface antigen; anti-HBc = antibody to hepatitis B core antigen; anti-HBs = antibody to hepatitis B surface antigen.

Table 3: **Anti-HBs titres of national servicemen immunized with three 10-µg doses of MSD plasma-based hepatitis B vaccine and followed up for 6 years**[a]

Anti-HBs titre (mIU/ml)	% +ve at post-vaccination follow-up after: 1 year (n = 226)	2 years (n = 278)	4 years (n = 284)	6 years (n = 190)
0	29.6	16.9	20.4	23.6
<10	9.0	18.3	21.5	21.5
10 to <100	30.5	35.3	29.9	32.5
100 to <1000	26.9	23.7	23.2	20.9
≥1000	4.0	5.8	4.9	1.6
Geometric mean titre	80.7	55.2	39.1	30.4

[a] See footnote *a*, Table 1.

Table 4: **Prevalence of hepatitis B virus markers among immunized and non-immunized national servicemen followed up for 6 years**

Follow-up:	Marker[a]	Prevalence among: Vaccinated	Unvaccinated
1 year	HBsAg	0/228[b] (0)[c]	
	Anti-HBc	2/226 (0.9)	Not tested
	Anti-HBs	159/226 (70.4)	
2 years	HBsAg	0/284 (0)	
	Anti-HBc	4/284 (1.4)	Not tested
	Anti-HBs	231/278 (83.1)	
4 years	HBsAg	2/293[d] (0.7)	
	Anti-HBc	8/293 (2.7)	Not tested
	Anti-HBs	226/284 (79.6)	
6 years	HBsAg	0/196 (0)	8/191 (4.2)
	Anti-HBc	4/196 (2.0)	40/191 (20.9)
	Anti-HBs	145/190 (76.3)	57/191 (29.8)

[a] See footnote *b*, Table 2.
[b] No. positive/No. tested.
[c] Figures in parentheses are percentages.
[d] Weakly positive; negative on repeat testing of a subsequent sample.

vaccine lots (*19*), or possibly to loss of potency, especially with a reduced dose, if the vaccine was not properly handled or was stored under suboptimal conditions. The higher immunogenicity of the vaccine could have accounted for the lower prevalence of anti-HBc (0.4–1.0%) and complete absence of HBsAg among the medical and dental students, compared with the national servicemen (0.9–2.7% anti-HBc positivity and 0.7% transient HBs antigenaemia).

A total of 45.6% of the national servicemen and 19% of the medical and dental students had no detectable anti-HBs or exhibited titres that were below the level considered to be protective (≥10 mIU/ml) (*20*) 5–6 years after vaccination. The relation between persistence of anti-HBs and duration of protection against HBV infection is still unclear. Low or undetectable levels of circulating anti-HBs may not necessarily indicate loss of protection. In high-risk adults, protection persists even when humoral antibody is no longer detectable (*20*). Moreover, when a booster dose was administered to healthy adults with undetectable anti-HBs 5–7 years after vaccination, an anamnestic response was elicited (*21*), implying that immunological memory persists. Thus, once an immune response has been induced by vaccination, it can be stimulated by exposure to the wild virus, with an active increase in anti-HBs during the early phase of the incubation period of the disease, thereby protecting against clinical illness or development of the carrier state. In an endemic setting repeated exposures to hepatitis B carriers could sustain or even boost the anti-HBs response without any serological evidence of infection (*22*).

In Singapore, a reduced dose (5 µg) of MSD plasma-based HBV vaccine is as effective as the standard child's dose (10 µg) in preventing the HBsAg carrier state in children up to 6 years of age (*23*). The present study also demonstrated the long-term efficacy of a reduced adult dose of MSD plasma-based vaccine (10 µg) in preventing the HBV carrier state in healthy young adults for at least 6 years after vaccination. The duration of protection conferred by this dose was no different from that of the standard 20-µg dose (*24*). Based on the results of local studies, reduced vaccine doses have been recommended for immunization of children and young adults in Singapore (*17*). In a recent national serological survey on vaccine-preventable diseases involving 878 healthy children and adults aged from 6 months to more than 45 years, HBsAg and anti-HBc were detected in 17 (5.6%) and 105 (34.8%), respectively, of 303 unvaccinated persons and in 2 (0.3%) and 41 (7.1%), respectively, of 575 vaccinated persons. However, both the HBsAg-positive vaccinees were over 45 years of age and not serologically tested prior to vaccination. This confirmed the adequacy of the reduced dose administered in a normal clinical setting in the prevention of the HBsAg carrier state in both children and adults in Singapore (*25*).

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Résumé

Immunogénicité et efficacité à long terme d'une dose réduite de vaccin antihépatite B dérivé de plasma chez le jeune adulte

Pour déterminer l'immunogénicité et l'efficacité d'une dose réduite (10 µg) de vaccin antihépatite B (Merck Sharp & Dohme) administrée à de jeunes adultes séronégatifs au début de l'étude puis au bout de 1 et 6 mois, nous avons suivi pendant 5 ans une cohorte d'étudiants en médecine et en art dentaire vaccinés et pendant 6 ans une cohorte de recrues du service national non vaccinées et vaccinées.

Parmi les étudiants, 81% avaient toujours des anticorps dirigés contre l'antigène de surface de l'hépatite B (anti-HBs) à un titre ≥10 mUI/ml au bout de 5 ans, à la fin de la période de suivi. Le titre moyen géométrique était passé de 412,6 mUI/ml au bout d'un an à 174,9 mUI/ml au bout de 5 ans. Bien que 0,4 à 1,0% des sujets aient été infectés, comme l'indique la présence d'anticorps dirigés contre l'antigène central de l'hépatite B (anti-HBc), aucun d'entre eux n'a été positif pour l'antigène de surface de l'hépatite B (HBsAg) pendant la période de suivi.

Dans le cas des recrues, les sujets vaccinés ont été revus au bout de 1, 2, 4 et 6 ans et les sujets non vaccinés seulement au bout de 6 ans. Les taux de séroconversion anti-HBs et les titres moyens géométriques des sujets vaccinés étaient beaucoup plus faibles que chez les étudiants, avec un taux de séroconversion anti-HBs de 55% (≥10 mUI/ml) et un titre moyen géométrique de 30,4 mUI/ml à la fin de la période de suivi. Une antigénémie HBsAg transitoire a été détectée chez 2 (0,7%) des 293 vaccinés au bout de 4 ans.

Au bout de 6 ans, l'HBsAg était absent chez l'ensemble des 196 vaccinés revus, mais a été détecté chez 8 (4,2%) des 191 recrues non vaccinées. Les taux correspondants pour l'anti-HBc étaient respectivement de 2,0% et 20,9%. Ces résultats confirment l'efficacité à long terme du vaccin antihépatite B dans la prévention du portage chronique de l'HBsAg chez le jeune adulte, même lorsqu'une dose réduite est administrée.

References

1. **Yvonnet B et al.** Low-dose hepatitis B vaccine immunization in children. *Lancet*, 1987, **1**: 169.
2. **Matsaniotis N et al.** Immunogenicity of low doses of hepatitis B vaccine in normal children. *Vaccine*, 1985, **3**: 297–299.
3. **Milne A et al.** Low dose hepatitis B vaccination in children. *New Zealand journal of medicine*, 1988, **99**: 47–49.
4. **Lelie PN et al.** Immunogenicity and safety of plasma-derived heat-inactivated hepatitis B vaccine (CLB): studies in volunteers at a low risk of infection with hepatitis B virus. *American journal of epidemiology*, 1984, **120**: 694–702.
5. **Goudeau A et al.** Immunogenicity of low dose (1.25 µg and 0.31 µg) hepatitis B vaccine. *Lancet*, 1984, **2**: 1091–1092.
6. **Papaevangelou G et al.** Safety and immunogenicity of a further reduced dose (10 mcg) of the hepatitis B vaccine. *Developments in biological standardization*, 1983, **54**: 209–215.
7. **Moyes CD et al.** Very-low-dose hepatitis B vaccine in newborn infants: an economic option for control in endemic areas. *Lancet*, 1987, **1**: 29–31.
8. **Oon CJ et al.** Evaluation of a low dose of hepatitis B vaccine given within a childhood immunization programme in Singapore. *Journal of infection*, 1986, **13**: 255–267.
9. **Deinhardt F.** Aspects of vaccination against hepatitis B: passive–active immunization schedules and vaccination responses in different age groups. *Scandinavian journal of infectious diseases*, 1982, **38**(suppl.): 17–23.
10. **Ayoola EA.** The immune response of healthy Nigerian adults to small doses of hepatitis B vaccine: comparison of 10 and 20 µg doses. *Journal of medical virology*, 1984, **13**: 223–225.
11. **Papaevangelou G et al.** Reduction of the dose of hepatitis B vaccine. *Journal of infection*, 1983, **7**(suppl.): 69–70.
12. **Papaevangelou G et al.** Immunogenicity of a 5-microgram dose of hepatitis B vaccine. *Journal of medical virology*, 1985, **15**: 65–69.
13. **Goh KT et al.** Comparison of the immune response of four different dosages of a yeast-recombinant hepatitis B vaccine in Singapore children: a four year follow-up study. *Bulletin of the World Health Organization*, 1992, **70**: 233–239.
14. **Guan R et al.** The immune response of low dose recombinant DNA hepatitis B vaccine in teenagers in Singapore. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1990, **84**: 731–732.
15. **Milne A et al.** Antibody response to recombinant, yeast-derived hepatitis B vaccine in teenage New Zealand children. *New Zealand journal of medicine*, 1988, **101**: 67–69.
16. **Guan R et al.** Immunogenicity of a low dose recombinant DNA hepatitis B vaccine in healthy adults in Singapore. *Asian Pacific journal of allergy and immunology*, 1989, **7**: 85–88.
17. **Goh KT et al.** The hepatitis B immunization programme in Singapore. *Bulletin of the World Health Organization*, 1989, **67**: 65–70.
18. **Goh KT et al.** The prevalence of hepatitis B virus markers in dental personnel in Singapore. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1988, **82**: 908–910.
19. **Ljunggren K et al.** Varying antibody response in hospital staff vaccinated against hepatitis B. *Scandinavian journal of infectious diseases*, 1988, **20**: 485–488.
20. **Hadler SC et al.** Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. *New England journal of medicine*, 1986, **315**: 209–214.
21. **Davidson M, Krugman S.** Recombinant yeast hepatitis B vaccine compared with plasma derived vaccine: immunogenicity and effects of a booster dose. *Journal of infection*, 1986, **13** (suppl. A): 31–38.
22. **Coursaget P et al.** Seven-year study of hepatitis B vaccine efficacy in infants from an endemic area (Senegal). *Lancet*, 1986, **2**: 1143–1145.
23. **Goh KT.** Hepatitis B virus infection in Singapore: epidemiology, prevention and control. In: Oon CJ, Aw SE, eds. *Proceedings of the International Symposium on Highlights of Viral Hepatitis and Hepatocellular Carcinoma Research, 22 November 1992, Singapore*. Singapore, Harper Press, 1993: 6–30.
24. **Wainwright RB et al.** Duration of immunogenicity and efficacy of hepatitis B vaccine in a Yupik Eskimo population. *Journal of the American Medical Association*, 1989, **261**: 2362–2366.
25. **Kane M.** Reduced doses of hepatitis B vaccines: is it a good idea? *Bulletin of the World Health Organization*, 1995, **73**(4): 529–530.