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A preliminary study of the immune response to locally produced recombinant hepatitis B vaccine in adult volunteers

**Khin May Oo, *Moh Moh Htun, *Win Maw Tun, *Kyin Kyin San, *Nyein Nyein, *Kay Khine Soe, *Tin Tin Myint & *Khin Pyone Kyi*

*Quality Control Division, Vaccine Plant
Department of Medical Research (LM)

The immune response to locally produced recombinant hepatitis B vaccine was studied on 17 male adult volunteers. All subjects were negative for the hepatitis B markers namely hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs). Three doses of recombinant hepatitis B vaccine were given at 0, 1, 2 month intervals by intramuscular (IM) route. Eleven subjects were given 3 vaccinations of 20μg per dose and 6 subjects were vaccinated with 40μg of recombinant hepatitis B vaccine as the initial dose and 20μg for the subsequent second and third doses. Blood samples were collected from each subject at 1, 2, 3 month intervals and tested for the presence of anti-HBs using the SD Anti-HBs ELISA 3.0, Standard Diagnostic, Korea. A known anti-HBs positive serum (400 m IU/ml) was used as the Working Standard. Anti-HBs was present in 23.5% of subjects one month after the first dose, 52.9% after the second dose and 88.2% after the third dose. The anti-HBs values ranged from 2 - 72 m IU/ml and protective anti-HBs levels (more than 10 m IU/ml) were achieved in 76.5% of the vaccinees at the end of 3 months. From this preliminary study, it could be concluded that the locally produced recombinant hepatitis B vaccine is immunogenic in human subjects.

INTRODUCTION

There are estimated 80 million HBV carriers in the South-East Asia Region, which are 7% of the regional population [1]. The World Health Organization have classified countries as high, intermediate and low endemic prevalence, depending on the carrier rate of the hepatitis B virus (HBV). Carrier rates of 8-20% are classified as high prevalence, 2-7% as intermediate and below 2% as low. Introduction of hepatitis B vaccine in the childhood immunization schedule is recommended for countries with the HBV carrier rate of more than 2%. In the South-East Asia region, only Nepal and Sri Lanka have prevalence rates below 2% [1]. Hepatitis B vaccine is effective in preventing HBV infections when it is given either before exposure or shortly after exposure [2].

Hepatitis B virus infection is hyper-endemic in Myanmar and is a major health problem in the country. A large-scale field study carried out in both lower and upper Myanmar had shown that 10.4% of the study population was seropositive for hepatitis B surface antigen (HBsAg) [3]. Subsequent studies carried out among different population groups had revealed HBsAg carrier rates of 10-12% [4].

A pilot scale production of plasma-derived hepatitis B was carried out at the Department of Medical Research (DMR) from 1990 to 1996, funded by the United Nations Development Programme and executed by the World Health Organization. Since 1997, a small-scale production of plasma-derived hepatitis B was carried out at the DMR with an annual production of approximately 100,000 pediatric doses. However, as the plasma-derived HB vaccine

was associated with the unwarranted fears by the public of acquiring blood-borne infections, production of an alternative hepatitis B vaccine was considered by the health authorities of the country.

In 2003, a recombinant hepatitis B vaccine produced from HBsAg expressed in the methylotrophic yeast *Hansenula polymorpha* was first produced at the Hepatitis B Vaccine Plant, Department of Medical Research (Lower Myanmar) with the technology transfer from the CJ Corporation, under the loan from the Economic Development and Cooperation Fund of the Republic of Korea.

Findings from the Phase 1 Clinical trial had established the safety of the candidate vaccine. This preliminary study was conducted to evaluate the immune response of the recombinant hepatitis B vaccine in adult human volunteers.

MATERIAL AND METHODS

Subjects

Seventeen adult volunteers from Ma Lit village in Hlegu Township who had participated in the Phase 1 clinical trial were further recruited for this study. The subjects were males and within the age range of 18-38 years. All the subjects were negative for hepatitis B surface antigen (HBsAg) and antibody to HBsAg (anti-HBs).

Candidate vaccine

Indigenous Recombinant hepatitis B vaccine produced from HBsAg expressed in methylotrophic yeast *Hansenula polymorpha.*

Methods

The subjects were divided into two groups: Group A and Group B.

(i) Group A

Group A comprised of 6 subjects and 40μg of recombinant hepatitis vaccine was given to each of the 6 subjects of Group A as the initial dose, in the deltoid region by intramuscular route according to the 0, 1, 2 month immunization schedule. 20μg of recombinant hepatitis vaccine was given for the second and third doses.

(ii) Group B

Group B consisted of 11 subjects and three doses of 20μg of recombinant hepatitis vaccine were given to each of the subjects of Group B in the deltoid region by intramuscular route, according to the 0, 1, 2 month immunization schedule.

Measurement of anti-HBs

Blood samples were collected from each subject at 1, 2, 3 months after the initial dose and tested for the presence of anti-HBs by an Enzyme-linked Immuno Sorbent Assay (SD Anti-HBs ELISA 3.0, Standard Diagnostic, Korea). Anti-HBs positive serum with a titer of 400 m IU/ml was used as the working standard.

*Interpretati*on

Seroconversion was defined as an anti-HBs concentration of more than 1 m IU/ml in a post-vaccination serum of an initially seronegative individual.

Sero-protection was interpreted as the presence of anti-HBs in concentrations of 10 m IU or more than 10 m IU/ml in a post-vaccination serum of an initially sero-negative individual.

RESULTS

Seroconversion rates of vaccinees from Group A were 16.6%, 50.0% and 83.3% one month after 1^{st}, 2^{nd} and 3^{rd} dose of vaccination, while the seroconversion rates of subjects from Group B were 27.2%, 54.5% and 90.9% after each dose. The overall seroconversion rate of the vaccinees of this study was 23.5%, 52.9% and 88.2% one month after the 1^{st}, 2^{nd} and 3^{rd} dose of vaccination (Table 1).

Table 1. Seroconversion rates of vaccinees at different time intervals

Time interval after1st dose	Group A (n=6)	Group B (n=11)	Total (%)
1 month	1 (16.6%)	3 (27.2%)	4 (23.5%)
2 month	3 (50.0%)	6 54.5%)	9 (52.9%)
3 month	5 (83.3%)	10 (90.9%)	15 (88.2%)

Although there was seroconversion, none of the vaccinees in Group A achieved sero-protective levels of anti-HBs at 1 month after the first dose, however, 33.3% and 50.0% of the vaccinees developed protective anti-HBs titers one month after the 2nd and 3rd vaccinations. The seroprotection rates of subjects from Group B were 27.2%, 54.5% and 90.9% one month after the 1st, 2nd and 3rd vaccinations. Overall, a total of 17.6%, 47.1% and 76. 4% of the vaccinees achieved seroprotection at 1, 2 and 3 months (Table 2). One subject from Group A and one from Group B failed to seroconvert at one month after the 3rd dose.

Table 2. Seroprotection rates of vaccinees at different time interval

Time interval after 1st dose	Group A (n=6)	Group B (n=11)	Total (%)
1 month	0 (0%)	3 (27.2%)	3 (17.6%)
2 month	2 (33.3%)	6 (54.5%)	8 (47.1%)
3 month	3 (50.0%)	10 (90.9%)	13 (76.4%)

Anti-HBs levels of 1- >50 m IU/ml were achieved in 83.3% of vaccines from the Group A and 90.9% from the Group B. In the two study groups, anti-HBS levels were present in 88.2% of the vaccinees (Table 3).

The seroconversion rate of Group A (6 cases), who received 40μg of vaccine as the initial dose was (83.3%), and seroprotection was achieved in 50% at 3 months after the first dose. In Group B, the seroconversion and seroprotection rates were both 90.9%. The overall sero-conversion rate was 88.2% out of which 76.5% achieved sero-protective levels of anti-HBs (Table 4).

Table 3. Anti-HBs titers in different groups (3 months after first dose)

Anti-HBs titer (m IU/ml)	Group A (n=6)	Group B (n=11)	Total (%)
1-9	2 (33.3)	0 (0)	2 (11.8)
10-30	1 (16.7)	2 (18.2)	3 (17.6)
31-50	1 (16.7)	2 (18.2)	3 (17.6)
> 50	1 (16.7)	6 (54.5)	7 (41.2)
Total	5 (83.3)	10(90.9)	15 (88.2)

Table 4. Seroconversion and seroprotection rates at 3 months after the first dose

Percentage	Group A (n=6)	Group B (n=11)	Total
Seroconversion (≥1 m IU/ml)	5 (83.3%)	10 (90.9%)	15 (88.2%)
Seroprotection (≥ 10 m IU/ml)	3 (50.0%)	10 (90.9%)	13 (76.5%)

DISCUSSION

In this study, the mean age of the subjects was 25.41 (SD 6. 20) years. The mean age of vaccinees allocated to group A was 26.83 (SD 6. 49) years while the mean age of the 11 subjects in Group B was 24.64 (SD 6. 20) years. Six subjects in Group A were given 40 μg of recombinant HB vaccine as the first dose followed by 20 μg for the second and third doses. Eleven subjects in Group B were given 3 doses of 20 μg of recombinant HB vaccine. The vaccines were given according to the 0, 1, 2 schedule.

Seroconversion rates (anti- HBs titer > 1m IU/ml) of vaccinees from Group A were 16.6%, 50.0% and 83.3% one month after 1st, 2nd and 3rd doses, while the sero-conversion rates of subjects from Group B were 27.2%, 54.5% and 90.9% at similar time intervals. One subject from Group A and one from Group B failed to seroconvert at one month after the third vaccination. The overall seroconversion rates in this

study were 23.5%, 52.9% and 88.2% one month after 1st, 2nd and 3rd vaccinations.

Although the seroconversion rates of Group B were slightly higher than those of Group A, there was no significant difference in the seroconversion rates between the groups, indicating that there was no dose-dependency in relation to the initial dose, although dose-dependency of yeast-derived HB vaccine was reported by Just and co-authors who found the geometric mean titer of anti-HBs levels in groups receiving 20 μg doses of yeast-derived vaccine were higher than in those administered the 10 μg doses [5].

The percentage of subjects responding to the vaccines with anti-HBs values above 10 m IU/ml are of greater importance than the overall seroconversion rates since it indicates protection against the hepatitis B virus infection. In this study, protective anti-HBs titers developed in 17.6%, 47.1% and 76.4% of the vaccinees, one month after the completion of the 1st, 2nd and 3rd doses of vaccination. Various authors had reported different seroprotection rates in studies using the 0, 1, 2, immunization schedule.

Seroprotection rates of 15.4%, 66.7% and 96.8% had been reported by Wiedermann *et al.* in a study population immunized with 3 doses of 20μg doses of yeast-derived hepatitis B vaccine [6]. In a study by Scheiermann *et al.* , the seroprotection rates were 20%, 70% and 100% in 220 medical students immunized with three 20μg doses of yeast-derived vaccine [7]. Isahak and co-authors reported the seroprotection rates of 7%, 79% and 100% in Malaysian medical students vaccinated with 3 doses of Engerix B (20μg) according to the 0, 1, 6 schedule [8].

Although the seroprotection rate of the candidate recombinant hepatitis B vaccine at one month after the first dose was comparable to the findings of other studies, the seroprotection rates following the second and third doses were found to be lower. Hepatitis B vaccines are highly immunogenic but have decreased immunogenicity associated with increasing age, obesity, smoking, presence of a chronic disease and male gender [9]. The relationship between diminished vaccine response and age, as well as other factors such as smoking and increased body mass had been observed by different authors [10, 11]. In the present study, the subjects were all males and more than 50% of them were smokers who might have contributed to the lower immunogenicity rates.

The time interval between the completion of the third dose and determination of anti-HBs was relatively short. Findings from many studies have shown that many seeming non-responders to hepatitis B vaccines must be considered as slow responders since the majority of apparent non responders develop antibodies at a later date [12] . If the anti-HBs testing was done at a later date, the two vaccinees who had failed to produce detectable levels of anti-HBs, might have seroconversion.

In conclusion, although the seroprotection rate was lower than that of other studies, the indigenous recombinant hepatitis B vaccine expressed in yeast cells of *Hansenula polymorpha* was found to be immunogenic and able to induce protective anti-HBs levels in the majority of the adult male volunteers.

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