Antibody-response to three recombinant hepatitis B vaccines: comparative evaluation of multicenter travel-clinic based experience

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Abstract

The immunogenicity of three currently used hepatitis B vaccines was compared in an unselected study population in an every day travel clinical setting. Five hundred and eighteen vaccinees received Engerix™-B (EB), 990 received Twinrix™ (TWX), and 366 were immunised with Gen-HB-Vax™ (GHB). Overall, 88.6% of the vaccinees, tested within the first 6 months after completion of the vaccination series, developed protective levels of anti-HBs (E > 10 mIU/ml). However, GHB recipients showed significantly lower seroprotection rates (SPR) than EB and TWX recipients (79.3% vs. 87.7% vs. 92.3%, P < 0.000001). GMTs for anti-HBs, tested within 6 months after the third vaccination, showed the lowest results in the GHB group, followed by EB and TWX (142 vs. 523 vs. 1008 mIU/ml, P < 0.000001). TWX vaccinees, however, showing a higher antibody decline rate than EB recipients within the first years after completion of the full immunisation course (30% vs. 25%; P = 0.0538). This study confirms an overall good immune response to the 20 µg-dose vaccine, in the course of a regular clinical setting. The significant difference in SPRs and GMTs to the 10 µg-dose vaccine, however, may influence future immunisation practices for the elderly. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Despite the success of recombinant hepatitis B vaccines in reducing the incidence of hepatitis B virus (HBV) infection [1], hepatitis B is still among the major health threats throughout the world, accounting for about 350 million people suffering from chronic HBV infection, and expected 105 million deaths owing to HBV-related liver disease [2].

The first human vaccine manufactured using recombinant DNA technology (Engerix™-B, SmithKline Beecham Biologicals, Rixensart, Belgium) has been widely investigated in more than 250 clinical trials [3] where it proved to be safe, efficacious and immunogenic [4–6]. Hepatitis B vaccination has been recommended for individuals in several well-recognised high-risk groups, including travelers [7].

Nevertheless, 5% of the vaccinated population do not respond adequately to currently used vaccination schedules. Apart from the known host related factors such as obesity, age, gender, smoking and concurrent illness [8,9], more recently, a certain genetic predisposition has been recognised as a possible cause for non-
low-responsiveness to vaccination in otherwise healthy subjects [10]. Moreover, it is not impossible that there are strains of HBV showing mutations and variants of HBsAg which could escape the immunity induced by the present vaccines [11–13].

However, most of the immunogenicity studies have been conducted under ideal, carefully controlled clinical trial situations in specifically selected healthy population groups. Immunogenicity results obtained with a vaccine under field conditions may be less, as controlled trials do not reflect reality in daily vaccine use [14].

The purpose of this present uncontrolled study under field conditions was to assess the immunogenicity of the currently used recombinant hepatitis B DNA vaccines in an unselected study population as it is present in an every day clinical setting in order to identify parameters that influence the response to hepatitis B vaccination under uncontrolled routine conditions.

2. Materials and methods

2.1. Vaccines

The hepatitis B vaccine, Engerix™-B (EB), and the combination hepatitis A/B vaccine, Twinrix™ (TWX), were produced by SmithKline Beecham Biologicals (Rixensart, Belgium). The other vaccine, Gen-H-B-Vax™ (GHB), was produced by Merck Sharp & Dohme (West Point, USA).

EB consisted of at least 20 μg yeast-derived recombinant HBV surface antigen adsorbed onto 0.5 mg of Al(OH)₃ in 1 ml volume. The adult formulation of the combination hepatitis A/B vaccine (TWX) contained 720 ELISA Units of formalin-inactivated hepatitis A virus (HAV) antigen and 20 μg DNA yeast-derived recombinant HBV surface antigen as separate adsorbates on 0.45 mg of aluminum hydroxide and aluminum phosphate in a 1 ml volume. GHB consisted of 10 μg yeast-derived recombinant HBV surface antigen adsorbed onto 0.5 mg aluminum salts in 1 ml volume.

2.2. Study population and design

This study was conducted in two independent outpatient institutions under routine practice in a travel clinic setting with healthy adolescents and adults who were seeking pre-travel immunisation advice. One of the study sites was at the Medical Faculty of the University of Vienna and one at a private travel clinic.

Participants were in total 1973 visitors (49% females, 51% males) aged between 6 and 80 yr (mean age 43.4 ± 15 yr). All individuals were booster vaccinated between January 1989 and December 1999. From these visitors 1874 were included in the evaluation (exclusion was due to missing information on schedule or missing Ab titers).

The choice of the type of vaccine was made with respect to the individual history of vaccinations and the eventually known HAV status of the vaccinee. Five hundred and eighteen individuals were immunised with Engerix™, 990 with TWX, and 366 with GHB. EB was available throughout the whole study period, whereas TWX and GHB were available for the last three years. There were no age limits. The only exclusion criteria were contraindications as mentioned in the prescribing information, including hypersensitivity to any component of the vaccine, or subjects with severe febrile illness. The vaccine dose was administered intramuscularly into the deltoid region according to a 0-, 1-, and 6- to 12-month schedule.

The demographic characteristics of the 1874 subjects included are given in Table 1. Post-vaccination serology testing was conducted for three years in the interval of 4 weeks to 5 yr following administration of the third dose of vaccine at the same laboratory for both participating centers.

2.3. Laboratory analysis

The immunogenicity was controlled by titer assessments (anti-HBs) 4 weeks to 5 yr following the third inoculation. In order to determine the anti-HBs titer, specimens were tested by a commercial enzyme immunoassay (AUSAB™ EIA, Abbott Laboratories) and expressed as milli-international units per milliliter (mIU/ml). Antibody titers above 1 mIU/ml were considered as indication of seroconversion. Persons with 10 mIU/ml or more of anti-HBs were defined as having protective levels against hepatitis B, according to ACIP standards [7,15,16]. Subjects showing HBs titers between 10 and 100 mIU/ml were referred to as low-responders. Only positive titers were used for geometric mean titer (GMT) calculation.

Table 1
Age and gender for the total sample and for the subgroup with titer examination within 6 months after booster for the different vaccination groups

<table>
<thead>
<tr>
<th></th>
<th>EB</th>
<th>TWX</th>
<th>GHB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sample</td>
<td>518</td>
<td>990</td>
<td>366</td>
</tr>
<tr>
<td>Age (mean ± S.D.)</td>
<td>42 ± 15</td>
<td>42 ± 15</td>
<td>47 ± 14</td>
</tr>
<tr>
<td>Gender (females)</td>
<td>43%</td>
<td>53%</td>
<td>49%</td>
</tr>
<tr>
<td>Subgroup tested within 6 months after booster</td>
<td>155</td>
<td>678</td>
<td>256</td>
</tr>
<tr>
<td>Age (mean ± S.D.)</td>
<td>43 ± 15</td>
<td>43 ± 15</td>
<td>47 ± 14</td>
</tr>
<tr>
<td>Gender (females)</td>
<td>48%</td>
<td>55%</td>
<td>49%</td>
</tr>
</tbody>
</table>
2.4. Statistical analysis

Anti-HBs antibody concentrations were log-transformed and subjected to analyses of variance, with age group or gender or season of vaccination as one factor and time after vaccination as the other factor. Additionally, for illustration the subgroup of vaccinees with titers obtained within 6 months after booster were subdivided into non responders (below 10 mIU/ml), low responders (between 10 and 100 mIU/ml) and responders (100 mIU/ml or more). These groups were evaluated according to age, gender and season of vaccination separately for each vaccine.

3. Results

All of the participants included in this study, completed the primary vaccination series and had post-vaccination serologic testing. The interval between the third inoculation and the serology testing for anti-HBs varied between 4 weeks and 5 yr. 58% (1089/1874) of the subjects were tested within 6 months after the third vaccination. For the 1089 persons evaluated, the mean time interval from receiving the third dose of vaccine to anti-HBs testing was 119.7 days (± 26.9 S.D.) for those who received TWX, 109.7 days (± 37.6 S.D.) for those who received EB, and 115.2 days (± 31.9 S.D.) for GHB vaccinees.

Of the 1089 participants tested within the first six months after completion of the vaccination course, 14.2% (155/1089) were vaccinated with EB, 62.3% (678/1089) with TWX, and 23.5% (256/1089) with GHB.

Demographic characteristics were similar among all three study groups (Table 1). Three different immunogenicity parameters were calculated: SPR (≥ 10 mIU/ml), low responder rate (< 100 mIU/ml), and GMT.

Overall, 88.6% (965/1089) of the vaccinees, who were tested within 6 months after completion of the vaccination series, developed protective levels of anti-HBs (≥ 10 mIU/ml). Among the 124 (11.4%) subjects who did not respond with protective anti-HBs titers (< 10 mIU/ml), 75 (60.5%) persons were older than 50 yr.

When vaccine performance was compared, GHB recipients showed significantly lower SPR to vaccination than EB and TWX recipients (79.3% vs. 87.7% vs. 92.3%, P < 0.000001). The vaccine specific differences in SPRs reached significance in the age group 30–50 yr (30–50 yr: P = 0.012; 50–60 yr: P = 0.047; > 60 yr: P = 0.0003) (Fig. 1). The same vaccine specific age-related trend could be observed regarding GMTs (P < 0.05 for age-groups 30–50 and > 60) (Table 2).

Overall, 9.5% (103/1089) had attained antibody levels between 10 and 100 mIU/ml. This group of vaccinees was referred to as low responders (TWX: 7.7%; EB: 12.3%; GHB: 12.5%; overall χ²: P < 0.000001; component for low-responders: TWX: P = 0.009).

Table 2

<table>
<thead>
<tr>
<th>Subgroup tested within 6 months after booster</th>
<th>EB</th>
<th>TWX</th>
<th>GHB</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>155</td>
<td>678</td>
<td>256</td>
</tr>
<tr>
<td>Total</td>
<td>523</td>
<td>1008</td>
<td>142</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15–30</td>
<td>1437</td>
<td>(647–3191)</td>
<td></td>
</tr>
<tr>
<td>30–50</td>
<td>738</td>
<td>1338</td>
<td>(993–1927)</td>
</tr>
<tr>
<td>50–60</td>
<td>451</td>
<td>359</td>
<td>(230–561)</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>96</td>
<td>298</td>
<td>(163–547)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>1115</td>
<td>(614–2024)</td>
<td>1564</td>
</tr>
<tr>
<td>Males</td>
<td>262</td>
<td>589</td>
<td>(419–827)</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>546</td>
<td>1444</td>
<td>(973–2144)</td>
</tr>
<tr>
<td>Summer</td>
<td>377</td>
<td>796</td>
<td>(526–1203)</td>
</tr>
<tr>
<td>Autumn</td>
<td>410</td>
<td>(168–999)</td>
<td>589</td>
</tr>
<tr>
<td>Winter</td>
<td>962</td>
<td>1704</td>
<td>(1099–2643)</td>
</tr>
</tbody>
</table>

When stratified by gender, the SPRs were 83.4% (431/517) in males and 93.4% (534/572) in females with a significant difference (P < 0.000001). Comparing gender specific GMTs, females showed significantly higher GMTs than males (P < 0.000001) (Table 2). GMTs for anti-HBs tested within 6 months after the third vaccination was 523 mIU/ml (95% CI 320–855) in the EB group, compared to 1008 mIU/ml (95% CI 817–1243) for those who received the TWX, and 142 mIU/ml (95% CI 98–206) in the GHB group (P < 0.000001) (Table 2). However, at year 2 after completion of the vaccination course, this difference was no longer statistically significant. Comparing the antibody...
Fig. 2. Comparison of EB (left) and TWX (right) cross-sections of anti-HBs antibody, 1 month to 5 yr following the third vaccination.

decline rate, we observed a decline rate of 25% in the EB group and 30% in the TWX group (P = 0.0538) (Fig. 2).

Analysing seasonality, GMTs after application of EB and TWX were significantly lower, if the third vaccination was performed in winter (947 mIU/ml) or autumn season (414 mIU/ml), compared to GMTs of subjects whose booster immunisation was performed in summer (428 mIU/ml) or spring (699 mIU/ml) (P = 0.002 for seasonality). The analysis of GHB GMTs, however, showed no significant differences (Table 2). The seasonal effects were reproducible for TWX in all study years (i.e. 1997–1999), for EB it was reproducible for all years except 1999 (i.e. 1995–1998). Analysing SPRs according to seasonality, no significant seasonal correlation could be observed.

4. Discussion

This comparative assessment of the immunogenicity of the currently licensed hepatitis B vaccines showed an overall high immune response in an unselected vaccine population as it is present in a routine travel clinic setting. Testing 1973 post-vaccination anti-HBs titers, we found, consistent with previous investigations [8,17,18], the most important non-modifiable determinant for non-response to vaccination increasing age and male gender. The results of the combined hepatitis A/B vaccine (TWX) and the monovalent 20 µg-dose hepatitis B vaccine (EB) highlight the good immunogenicity of the 20 µg-dose of recombinant hepatitis B vaccines as serum levels ≥10 mIU/ml are considered to be protective against disease [14,15]. TWX, however, turns out to be the most immunogenic of the three vaccines, most significant in subjects > 60 yr old. Over the age of 60 TWX is clearly more immunogenic than EB. So the dose effect (20 µg vs. 10 µg) does not seem not to be the only factor involved. It is known, that in Austria the prevalence of anti-HAV lies between 60% and 80% in subjects older 60 yr of age [19]. In such cases the naïve anti-HBs response may then have benefited from the memory response (T-help) towards HAV. Although, this is rather speculative, this point needs further elucidation as we did not examine the HAV status prior to vaccination in all vaccinees except in cases of a clearcut history of clinical hepatitis A disease. In contrast, the immunogenicity results of the 10 µg-dose vaccine (GHB) showed significantly higher rates of non-responders (< 10 mIU/ml) in vaccinees older than 40 yr of age. This may be because of the lower content of antigen in the adult dose of GHB compared to EB. The difference in SPR between the two dosages increases with higher age. In the age-group > 60 yr, GHB vaccinees showed a 3.5 times higher rate of non-responders than TWX recipients, and 1.6 times higher rate than EB vaccinees. This observed difference remains, even when controlled for gender and age.

Several published studies, comparing the immunogenicity of the two dosages, showed that the 20 µg-dose produced significantly higher anti-HBs GMT than the 10 µg-dose of GHB, but no statistically significant difference in SPR were observed so far [17,20–23]. One has to keep in mind, that the results of these studies were obtained in carefully monitored clinical trial situations with either healthy study population < 40 yr of age, limited number of subjects or selected cluster of vaccine recipients (newborns, children, homogenderuals, adolescents). In these studies a selected cohort of subjects were followed at fixed times without taking into account common variables encountered in clinical practice. The mean age of this unselected cohort of vaccinees of the present study was 43.4 yr (6–80), reflecting the mean age of real-life population of vaccinees with no age limits. Moreover, the large cohort of 1973 vaccinees in this study exceeded the number of subjects in controlled immunogenicity studies.

Indeed, it is likely that some of the non-responders are in fact primed after a series of vaccinations even without detectable antibodies. Chiaramonte et al. [18] speculate that one might have developed cell-mediated immunity without humoral response which may develop later after booster immunisation. However, one can not rely on this speculative protection in non-responders. Consequently, every effort needs to be made to ensure seroconversion ≥10 mIU/ml, when necessary through the administration of additional booster doses. These results confirm recommendations for adults for routine post-vaccination assessment of HBs status earliest one month after the primary vaccination or booster in order to identify vaccinees with HBs-antibody levels < 10 mIU/ml.

Following the consensus statement of the European Consensus Group on Hepatitis B Immunity, there is no need for booster vaccination after a successful primary
vaccination series in healthy individuals, as the maintenance of HbsAg-specific memory confers protection to clinically breakthrough infection even in the absence of detectable antibodies [24]. In fact, the influence of age on the immune response has been proven by various studies [25–28]. Although we have not presented any experimental data for an influence of a decrease of memory cells on an impaired hepatitis B immune response in elderly people and although such deliberation might have no direct influence on the European Consensus Group Statements, further studies might be needed to find out if the hepatitis B booster vaccination should be suspended for all age groups.

Results of our study confirm the significant effect of gender on the immune response, in terms of lower SPRs and GMTs in males, elicited after 3-dose schedule. This finding has also been found in other immunogenicity studies [29,30]. In this study, the rate of non-responders among males was about 3 times higher than among female vaccinees in all three vaccine groups.

Typically, the level of anti-HBs titers do gradually decline with time, with the greatest reduction occurring during the first year after vaccination [31–38]. In our study, comparing vaccine-specific cross-sections of post-vaccinal anti-HBs levels of vaccinees depending on the time between the third vaccination and the time of antibody testing, we observed the same phenomenon as found in the aforementioned longitudinal profiles of antibody persistence. The cross-sections of both of the 20 μg-dose groups show a more rapid decay within the first 1.5 yr than during the following time. TWX vaccinees, however, show a higher anti-HBs titer 1–6 months after completion of a full immunisation course than EB recipients, followed by a higher decline rate, starting one month after the third administration, resulting in converging curves after two years after completion of the immunisation course. These cross-sectional curves of antibody decline cannot be directly compared to the results of longitudinal observations as we did not measure the individual antibody levels over a period of time but investigated a cross-section of a representative unselected cohort with intervals varying between 1 month and 5 yr, between the third vaccination and time of antibody testing. Our cross-sectional findings, however, do not directly confirm earlier studies that antibody decline rates after application of different hepatitis B vaccines might be comparable [39,40].

Analysing the seasonality of the third inoculation of TWX or EB, we observed significantly higher levels of HBs antibody when the booster administration was performed in winter or spring season than during the warmer part of the year, indicating a seasonal correlation of the immunogenicity of booster vaccination. Although, this effect is of rather little practical use, it appears to be an objective finding as the seasonal differences were reproducible when single years are considered except for EB for the last study year (1999). The reason for the different effects in this single year might be seen in the fact that the majority of vaccinees in 1999 were immunized with TWX. One can only speculate, however, about the reasons for such a phenomenon which has so far only been observed with live vaccines such as the oral polio vaccine [41]. The reason for no significant seasonal correlation of GMTs of GHB vaccinees might be due to the overall low GMTs in this group which may mask such an effect.

In conclusion, our study confirms the overall good immune response to the currently licensed 20 μg-dose recombinant DNA derived hepatitis B vaccines after a full vaccination course. Comparing the combined vaccine with the monovalent 20 μg vaccine, higher postvaccinal anti-HBs titers can be observed in the combination vaccine group, though, followed by equal cross-sectional antibody levels two years after completion of the full immunisation cycle. The 10 μg-dose hepatitis B vaccine (GHB), however, shows a significantly lower immunogenicity with advancing age, leading to the conclusion that the higher-dose vaccine might be more effective in the elderly.

References
