

IMMUNOGENICITY OF HBV VACCINE DURING STATED SHELF-LIFE

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Abstract. Thiomersal has been used as preservative in multi-dose vials of hepatitis B vaccine (*Engerix*TM-B). Due to safety concerns, thiomersal was replaced with 2-phenoxyethanol (2PE) as preservative in multi-dose vials. The potency of 2PE preserved hepatitis B vaccine multiple use vials was measured over the shelf-life in terms of immunogenicity, reactogenicity and safety. This single-blind, randomized study was conducted with the assistance of employees of GlaxoSmithKline Biologicals, makers of the *Engerix*TM-B vaccine. Four hundred twenty subjects aged ≥ 18 years were randomized to receive three doses (0, 1, 6 months) of 2PE preserved hepatitis B vaccine kept on the shelf <12 months (2PE New group), 2PE preserved hepatitis B vaccine kept on the shelf >18 months (2PE Old group), or thiomersal preserved hepatitis B vaccine [HBV(Thio) group]. Anti-HBs was measured by GlaxoSmithKline Biologicals post-vaccination; the reactogenicity and safety of the vaccines were assessed. Protective anti-HBs levels (≥ 10 mIU/ml) were measured one month after dose 3. The results showed protective levels in 86.8% (2PE New), 89% (2PE Old) and 95.3% [HBV(Thio)]. There was no difference detected between the 2PE New and 2PE Old groups in terms of anti-HBs seroprotection rates and geometric mean concentrations one month after dose 3. However, both 2PE groups had significantly lower seroprotection rates than the HBV(Thio) group and the number of non-responders was higher in the 2PE groups than in the Thio group. A antibody response rates over time were similar between the 2PE New and Old groups. The reactogenicity profiles were acceptable and the ranges were similar for each group. The shelf-life of the vaccines had no impact on immunogenicity or reactogenicity and 2PE preserved hepatitis B vaccine can be considered stable over time.

Key words: HBV vaccine, immunogenicity, shelf-life, preservative

INTRODUCTION

Hepatitis B is a worldwide health problem and an important cause of acute

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and chronic liver disease (WHO, 2004). According to World Health Organization (WHO) estimates, two billion people globally have serological evidence of past hepatitis B infection and approximately 360 million are estimated to be chronically infected with the virus (WHO, 2000, 2004). Of these, 15-25% are expected to die from liver disease, cirrhosis or primary hepatocellular carcinoma (WHO, 2000, 2004). Pre-

vention of infection through vaccination is the most effective strategy to reduce global morbidity and mortality due to hepatitis B (Zanetti *et al*, 2008).

*Engerix*TM-B, GlaxoSmithKline Biologicals' recombinant DNA hepatitis B vaccine, contains hepatitis B surface antigen (HBsAg) from genetically engineered yeast (*Saccharomyces cerevisiae*). Immunogenicity, safety and efficacy of *Engerix*TM-B have been demonstrated (Poovorawan *et al*, 1990, 1992; Keating and Noble, 2003). Thiomersal has been used as a preservative in multi-dose vials of *Engerix*TM-B. Thiomersal is an organomercurial preservative commonly used in vaccines and other parenteral medications. Multi-dose vials of hepatitis B vaccine have lower manufacturing costs, require less storage space when compared to monodose vials, and can be used in settings where cold storage capacity is limited (EMEA, 2001; Clements and Wesselingh, 2005). However, unlike monodose vials that are discarded immediately after single use, multi-dose vials are used more than once after the vials are opened. Thus, preservatives are added to multi-dose vaccine vials to prevent microbial contamination between use during the vaccine's shelf-life. Thiomersal was previously the preservative of choice. The thiomersal present in *Engerix*TM-B was a result of the purification process (residual amount of <2 µg/20 µg HBsAg) and was also added as a preservative to the final vaccine.

In response to safety concerns about thiomersal in human vaccines, regulatory agencies called for removal of thiomersal from these products (AAP, 1999; EMEA, 1999). All *Engerix*TM-B vaccines are now manufactured in monodose vials and are preservative free (CDC, 2000). However, at a time when multi-dose preparations were still in use, thiomersal was replaced with

another preservative, 2-phenoxyethanol (2PE), also commonly used in human vaccines.

When stored over a long period of time, vaccines are known to gradually lose their potency and have lower immunogenicity (WHO, 1998). This study evaluated the immunogenicity, reactogenicity and safety of two formulations of multi-dose *Engerix*TM-B; one containing thiomersal and the other containing 2PE as preservative. The 2PE containing vaccine was divided into two lots: one lot on the shelf <12 months and one lot on the shelf >18 months. We report here immunogenicity of the 2PE-containing vaccine at different lengths of time on the shelf.

MATERIALS AND METHODS

Study design and subjects

This was a single-blinded, randomized (1:1:1) study (eTrack study ID: 103860/283) conducted in the Philippines between May 2003 and March 2004 in accordance with the Good Clinical Practice and Declaration of Helsinki (1996 version). The study protocol was approved by the local ethics committee and written informed consent was obtained from all study participants.

Subjects aged ≥18 years were enrolled and randomized into one of the three groups: the 2PE New group (vaccinated with a 2PE-containing hepatitis B vaccine on the shelf <12 months), the 2PE Old group (vaccinated with a 2PE-containing hepatitis B vaccine on the shelf >18 months), the HBV(Thio) group (vaccinated with thiomersal-containing hepatitis B vaccine). Subjects received three vaccine doses at 0, 1 and 6 months. Enrolled subjects were seronegative for HBsAg, anti-hepatitis B core antigen (anti-HBc) antibodies and anti-hepatitis B surface antigen

(anti-HBs) antibodies. Subjects were excluded from the study if they were pregnant, immunocompromised, had a history of hepatitis B or had previously received hepatitis B vaccination.

Vaccines

The vaccine was in a multi-dose (10 doses) vial. Each 1 ml dose contained 20 µg of HBsAg. The 2PE formulations each contained 5 mg of 2PE and <2 µg of thiomersal, while the HBV(Thio) formulation contained 50 µg of thiomersal.

Assessment of immunogenicity, reactogenicity and safety

Blood samples were collected at Months 1, 2, 6 and 7 to measure anti-HBs antibodies. The assessment was done at GSK Biologicals central laboratory (enzyme immuno assay, AUSAB[®] EIA/Abbott laboratories).

Solicited local (pain, redness and swelling at the injection site) and general (fatigue, fever, gastrointestinal symptoms and headache) symptoms were recorded for 4 days (Day 0 to Day 3) after each vaccine dose. Symptom intensity was graded on a scale of 0-3. Grade 3 redness and swelling was defined as injection site diameter ≥ 50 mm and grade 3 fever as an axillary temperature $>39^{\circ}\text{C}$. For all other symptoms, grade 3 was defined as a symptom that prevented normal everyday activities. Unsolicited symptoms were recorded for 31 days (Day 0 to Day 30) after each vaccine dose and serious adverse events (SAE) were recorded throughout the study period.

Statistics

The immunogenicity analysis was performed according-to-protocol (ATP) cohort that included all eligible subjects who complied with the protocol-defined procedures. The analysis of solicited symptoms was conducted on the ATP

safety cohort, which included all subjects who had not received a vaccine forbidden by the protocol. The analysis of unsolicited symptoms and SAEs was conducted on the total vaccinated cohort.

Subjects with an anti-HBs antibody concentration ≥ 3.3 mIU/ml were considered to be seropositive and those with a concentration ≥ 10 mIU/ml were considered seroprotected. Anti-HBs seroprotection rates and geometric mean concentrations (GMCs) were tabulated with 95% confidence intervals (CI). Non-inferiority between the 2PE New group and the HBV(Thio) group (primary study objective) was concluded if the lower limit of the asymptotic 95% CI on the group difference [2PE New minus HBV(Thio)] in terms of seroprotection rates was $>10\%$. Similar non-inferiority calculations in terms of seroprotection rates were performed for the group difference between 2PE Old and HBV(Thio) groups and between 2PE Old and 2PE New groups. Exploratory analyses assessed the non-inferiority of the 2PE New to the HBV(Thio) group, non-inferiority of the 2PE Old to the HBV(Thio) group and non-inferiority of the 2PE Old to the 2PE New group in terms of anti-HBs antibody concentrations obtained at one month after dose 3 by computing the 95% CI for the pair-wise GMC ratios among the three groups using a one-way ANOVA model. If the 95% CI of the GMC ratios between the groups under consideration contained the value 1, then non-inferiority was established.

RESULTS

Subjects

A total of 420 subjects were enrolled and randomized: 140 in the 2PE New group, 141 in the 2PE Old group and 139 in the HBV(Thio) group. Twenty-five sub-

Table 1
Seropositivity, seroprotection rates and GMC (ATP cohort for immunogenicity).

Group	Timing	N	S+		SP		GMC (95% CI)
			n	% (95% CI)	n	% (95% CI)	
2PE New	One month post-Dose 3	129	117	90.7 (84.3-95.1)	112	86.8 (79.7-92.1)	2,163.7 (1,388.9-3,370.6)
2PE Old	One month post-Dose 3	127	115	90.6 (84.1-95.0)	113	89.0 (82.2-93.8)	2,621.0 (1,701.9-4,036.6)
HBV(Thio)	One month post-Dose 3	128	123	96.1 (91.1-98.7)	122	95.3 (90.1-98.3)	2,108.3 (1,425.3-3,118.7)

N, number of subjects with available results

S+, seropositivity for anti-HBs antibodies (≥ 3.3 mIU/ml)

SP, seroprotection for anti-HBs antibodies (≥ 10 mIU/ml)

n, number of subjects with concentration within the specified range

%, percentage of subjects with concentration within the specified range

95% CI, 95% confidence interval

GMC, geometric mean concentrations in mIU/ml

jects withdrew during the study. Two withdrew due to non-serious adverse events (prolonged menses and menstrual-like bleeding) and two withdrew due to SAEs (nervous breakdown, severe headache and numbness of extremities). None of the adverse events leading to withdrawal were considered by the investigator to be related to vaccination. Two subjects were eliminated from the ATP safety cohort ($n = 418$) due to randomization failure and the vaccine not administered as per the randomized group. Additionally, nine subjects were eliminated from ATP immunogenicity cohort ($n = 409$) due to non-compliance with blood-sampling intervals.

Subjects in the three groups were comparable in terms of age, gender and race. The mean age of the subjects was 33.3 years (standard deviation 11.5 years); 65.3% were female, 99.5% of subjects were of Southeast Asian origin.

Immunogenicity

The anti-HBs antibody seroprotection

rate one month after dose 3 was 86.8% (95%CI 79.7, 92.1) in the 2PE New group, 89.0% (95%CI 82.2, 93.8) in the 2PE Old group and 95.3% (95% CI 90.1, 98.3) in the HBV(Thio) group. One month after dose 3 the antibody GMC was 2,163.7 mIU/ml (95% CI 1,388.9, 3370.6) in the 2PE New group, 2,621.0 mIU/ml (95% CI 1,701.9, 4,036.6) in the 2PE Old group and 2,108.3 mIU/ml (95% CI 1,425.3, 3,118.7) in the HBV(Thio) group. There were 12 subjects each in the 2PE New and 2PE Old groups and five in the HBV(Thio) group who did not respond to the three doses of hepatitis B vaccine (Table 1). If the non-responders were excluded, the anti-HBs antibody concentrations in the three groups appeared similar (Fig 1).

Non-inferiority between the 2PE New and the HBV(Thio) groups could not be established since the pre-defined non-inferiority criterion was not met (group difference = -8.5% (95%CI -16.0, -1.6)). Non-inferiority between the 2PE Old and HBV(Thio) was also not established since

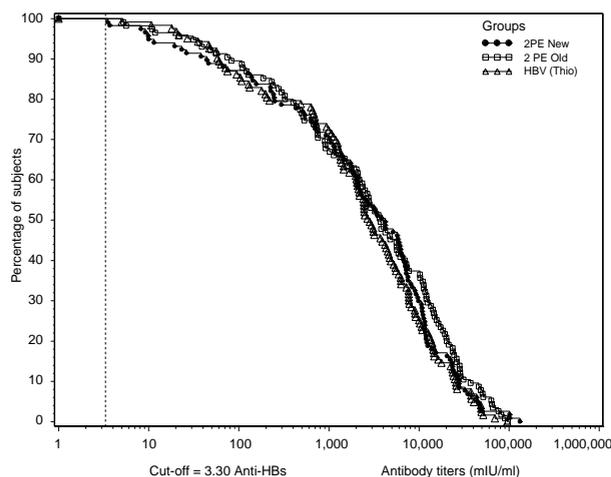


Fig 1—Reverse cumulative curve for anti-HBs concentrations after excluding subjects seronegative at one month after dose 3 of the hepatitis B vaccine (according to the protocol immunogenicity cohort).

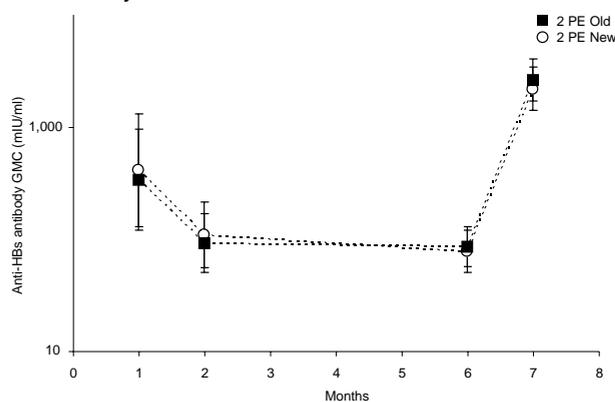


Fig 2—Anti-HBs geometric mean antibody concentrations (GMC) in the 2PE New (open circles) and 2PE Old (squares) groups (according to the protocol immunogenicity cohort). Months 1, 2 and 7, one month after doses 1, 2 and 3 respectively, Month 6 is prior to dose 3. Error bars = 95% CIs.

the pre-defined non-inferiority criterion was not met (group difference = -6.3% (95%CI $-13.6, 0.3$)). However, non-inferiority between the 2PE New and 2PE Old groups in terms of anti-HBs seroprotection rates was established (group difference = 2.2% (95% CI $-6.0, 10.4$)). Exploratory

analysis showed no difference between groups in terms of the post-dose 3 GMC (95% CI on the group ratios included the value 1 for all comparisons; data not shown). The kinetics of the antibody response over time was similar between the 2PE New and 2PE Old groups (Fig 2).

Reactogenicity and safety

Pain at the injection site (9.1% to 12.9% of doses) and fatigue (3.9% to 5.6% of doses) were the most frequently reported local and general symptoms, respectively, for all three groups (Fig 3). Grade 3 local and general symptoms were infrequently reported ($\leq 0.2\%$ of doses of all groups). During the 31-day follow-up period, $\leq 2.7\%$ of doses were followed by unsolicited symptoms. Eight doses were followed by at least one unsolicited adverse events that was considered to be causally related to vaccination. All were reactions at the injection site (one in the 2PE new group, two in the 2PE Old group and five in the HBV(Thio) group). Five subjects (one each in the 2PE Old and New groups and three in the HBV(Thio) group) reported 6 SAEs during the study. None were determined by the investigator to be causally related to vaccination.

DISCUSSION

Two lots of 2PE-containing hepatitis B vaccine differing only in the time on the shelf, gave similar immunogenicity when used for primary vaccination of healthy adolescents against hepatitis B. Although non-inferiority of the 2PE New group to the HBV(Thio) group in terms of anti-HBs

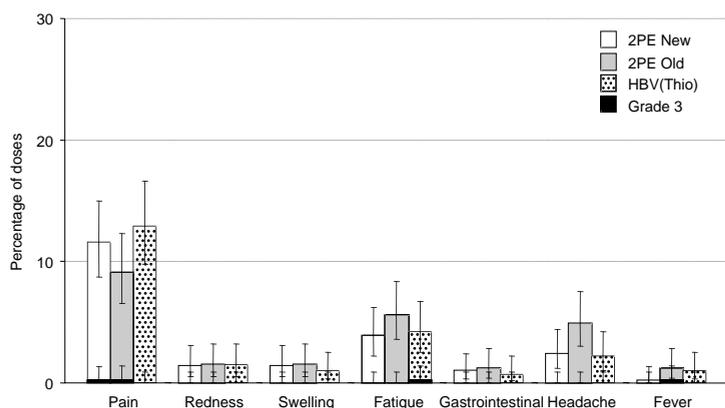


Fig 3—Solicited local and general solicited symptoms according to the protocol safety cohort. $N = 415$ doses in the 2PE New group, $N = 408$ doses in the 2PE Old group and $N = 403$ doses in the HBV(Thio) group.

seroprotection rate could not be demonstrated; therefore, the HBV(Thio) group had a significantly higher seroprotection rate than the 2PE groups. The two groups also differed in the number of non-responders [12 in the 2PE New group and 5 in the HBV(Thio) Group] to the hepatitis B vaccine. Another study found the thiomersal free formulations of hepatitis B vaccine had similar seroprotection rates when compared to thiomersal containing hepatitis B vaccine (Van Damme *et al*, 2009).

An exploratory analysis showed no difference between the 2PE-containing groups in terms of anti-HBs seroprotection rates or GMCs one month after dose 3. The kinetics of the responses over the 3-dose vaccination course were within the same range for both groups (Fig 2). Data from the WHO shows hepatitis B vaccines are generally stable for up to four years when stored at 2-8°C (WHO, 2006). Results from the present study also suggest the shelf-life of the 2PE preserved hepatitis B vaccine did not have an impact on vaccine immunogenicity for >18 months.

The vaccines had a clinically acceptable safety and reactogenicity profile. Nei-

ther removal of thiomersal from the vaccines, or the shelf-life of the 2PE-containing vaccines appeared to influence the incidence of adverse events after vaccination. Grade 3 symptoms after vaccination occurred infrequently and were reported after no more than 0.2% of doses. No SAEs reported during the entire study period were considered by the investigator to be related to vaccination. These results are in line with a study conducted with another preservative-containing hepatitis B vaccine

(Rebedea *et al*, 2006).

The immunogenicity of 2PE-containing hepatitis B vaccine is stable when stored for <12 months or >18 months, in line with data from the WHO (WHO, 2006). The multi-dose hepatitis B vaccine used in this study gave acceptable immunogenicity and reactogenicity. Complying with the recommendations of regulatory authorities to phase out the use of preservatives from vaccines (AAP, 1999; EMEA, 1999), GlaxoSmithKline Biologicals has discontinued production of multi-dose versions of *Engerix*TM-B vaccine and has removed all preservative from monodose *Engerix*TM-B formulations.

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Bernard Hoet, Hans L Bock and Karthik Srinivasa were employees of GlaxoSmithKline Biologicals at the time the study was conducted and during manuscript preparation. Nina G Gloriani declared she has no conflicts of interest. The laboratory tests were all performed by GlaxoSmithKline Biologicals.

Engerix-B is a trademark of the GlaxoSmithKline group of companies.

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