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Immunogenicity of the Brazilian hepatitis B vaccine in adults

ABSTRACT

OBJECTIVE: To evaluate the immunogenicity and safety of a novel hepatitis B vaccine, after increasing antigen concentration to 25 μg, in comparison to the reference vaccine.

METHODS: Single-blinded randomized trial comparing VrHB-IB (Instituto Butantan) and the reference vaccine (Engerix B®, Glaxo Smith Kline). Volunteers aged 31 to 40 years were randomized to either experimental (n=216) or control (n=203) groups, and were given three doses of vaccine. The first dose was administered upon recruitment, and the second and third doses 30 and 180 days later, respectively, between 2004 and 2005. Blood samples were collected for analysis before randomization and after the second and third doses. Active search for adverse effects was performed in the first five days after vaccination. Differences were evaluated using chi-square and Fisher's exact tests, with a 5% significance level.

RESULTS: No severe adverse effects were observed. Seroprotection was confirmed in 98.6% (213/216) of volunteers in the experimental group and 95.6% (194/203) of those in the control group. Geometric mean titers were 12,557 and 11,673, respectively.

CONCLUSIONS: The Brazilian vaccine was considered to be equivalent to the reference vaccine and its use is recommended for adults.

DESCRIPTORS: Hepatitis B Vaccines. Efficacy. Hepatitis B, prevention & control. Controlled Clinical Trial.

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INTRODUCTION

Hepatitis B is still a global public health problem. The World Health Organization estimates that roughly 2 billion people have been infected by the hepatitis B virus, of which 350 million are chronically infected.[a] Chronically infected patients are at high risk of death from hepatic cirrhosis and liver cancer. Annual hepatitis B-associated mortality is estimated at 600 thousand deaths.[b]

In Brazil, areas of high and moderate hepatitis B endemicity include the Western Amazon, Western and Southern Paraná, Western Santa Catarina, the Jequitinhonha valley in the state of Minas Gerais, and certain areas in the state of Mato Grosso. Low endemicity areas include the remainder of the Brazlian South and Center-West, as well as the Northeast and Southeast. Recent studies have shown an impressive decline in prevalence of chronic hepatitis B infection in the Western Amazon, possibly as a result of vaccination strategies. Preliminary results of the National Survey of Viral Hepatitides have provided estimated prevalences of 0.11% and 0.5% among 10- to 19-year-olds and 20- to 69-year-olds, respectively, in the Northeast Region and of 0.17% and 0.75% in these same age groups in the Center-West Region.[1,4,5,16]

Despite such reductions in prevalence, however, hepatitis B remains an important public health concern in Brazil.

In the past three decades, several advancements have been made in the development of vaccines protective against hepatitis B, from the use of 22 nm non-infectious viral particles purified from the plasma of asymptomatic HBV carriers to the large-scale production of vaccines using recombinant DNA technology. Much has been published on the safety, immunization schemes, and efficacy of this vaccine, and current formulations are safe, immunogenic, and capable of preventing the large majority of cases of infection, thus drastically reducing HBV-related mortality.[6]

The efficacy of the recombinant HBV vaccine was initially demonstrated in 1980, in the classical study by Szmuness et al,[14] which showed reduced incidence of HBV infection in a population of men who had sex with men. The hepatitis B vaccine is highly immunogenic and protective against HBV infection. A response is considered to be protective when the vaccine elicits the formation of antibodies to HBsAg (anti-HBs) at a level equal to or greater than 10 mUI/ml as determined by immunoenzymatic assay. A complete series of three or four doses of hepatitis B vaccine is capable of eliciting a protective response in over 90% of healthy adults and over 95% of healthy children and adolescents. Though vaccination schemes may vary, the usual recommendation consists of either three doses administered at zero, one, and six months, or four doses administered at zero, one, two, and 12 months. Vaccination induces antibody titers in the order of 1,000 to 3,000 mUI/ml in adults and higher than 5,000 mUI/ml in children.[15]

The initial response to vaccination decreases with increasing age. Among healthy children, adolescents, and young adults (20-39 years), protective immunization is generally higher than 90%, falling to 70% among adults aged 50-59 years and to 50% among those 60 years or older. Other factors influencing the immunogenicity of the vaccine include smoking, obesity, and immunosuppressive diseases, including diabetes mellitus, corticosteroid treatment, chronic renal insufficiency, and HIV infection.[8,12] Still, a proportion of healthy individuals, ranging from 2.5% to 5%, do not respond satisfactorily to HBV vaccination. Such individuals are considered to be unresponsive to the hepatitis B vaccine.[3,11]

The Food and Drug Administration recognized the safety of the hepatitis B vaccine based on an evaluation of 12 million doses of vaccine administered to infants. Side-effects are similar to those of other licensed vaccines. Pain and hyperemia at the injection site are the most frequent adverse effects (15%-20%), and are likely to be caused by the vaccine's adjuvant, aluminum hydroxide.[10,14] Approximately 15% of vaccinated individuals experience one or more mild and self-limiting systemic symptoms, such as cephalea, fever, and/or fatigue, usually 24 to 28 hours after vaccination.[13]

The Instituto Butantan (IB) in São Paulo began the production of a hepatitis B vaccine using recombinant DNA technology (VrHB-IB). This vaccine was initially formulated using 20 μg of recombinant HBsAg, with aluminum hydroxide as an adjuvant. Preliminary studies in healthy adult volunteers, using 20 μg of antigen per dose in a zero, one, and six month immunization scheme, showed that the IB vaccine did not induce significant adverse effects and led to seroconversion in 95.3% of subjects.[2] It was later found that the IB formulation was not as effective in eliciting an immune response in subjects older than 45 years, with 70% seroconversion among these subjects in comparison with 100% in the 18-25 years age group. Moreover, given the differences in geometric mean antibody titer after vaccination between the 20 μg dose of the test vaccine and the control vaccine, the authors suggested the need for increasing antigen concentration in the vaccine.[7]

As a complement to previous studies, the Brazilian Ministry of Health sponsored a multicenter trial aimed at evaluating the immunogenicity and safety of the IB vaccine in different age groups. This trial used Engerix B®, produced by Glaxo Smith Kline, as a reference HBV vaccine. This vaccine is obtained using the same genetic engineering technology as the IB vaccine, and is acknowledgedly safe and highly immunogenic.

The multicenter trial concluded that the IB vaccine was safe and elicited a good immune response in children, adolescents, and young adults. However, the response among adults aged 31 to 40 years was unsatisfactory, with 79.9% of subjects becoming seroproteced, compared to 92.5% using Engerix B®.[9]

It was therefore concluded that the IB vaccine should be improved before it could safely be used in adults. Instituto Butantan thus reformulated the vaccine as an attempt to increase its immunogenicity. HBs antigen concentration was increased from 20 to 25 μg/dose, which yielded an eletrophoretic profile similar to that of Engerix B®.

The aim of the present study was to analyze the immunogenicity of the reformulated VrHB-IB in healthy adults, in comparison with the reference vaccine.

METHODS

We carried out a single-blinded, controlled, randomized trial. Volunteers, aged 31 to 40 years, were recruited from among the personnel of the City of Sao Paulo Metropolitan Police, in the municipality of São Paulo, Southeastern Brazil. Subjects were divided into two groups: the experimental group received VrHB-IB in the 25 μg/dose formulation, and the control group received the vaccine considered as standard, Engerix B® at 20 μg/ml, from Glaxo Smith Kline. Each participant received three doses of one of the vaccines, at days zero, 30 or later, and 180 or later. Blood samples were collected before the first and third doses, and 30 days after the third dose. Beginning on the fifth day after each dose, subjects were contacted to collect information on adverse effects. Fieldwork was carried out between the second semester of 2004 and the first semester of 2005.

VrHB-IB was provided by the Brazilian Ministry of Health's National Immunization Program, and Engerix B® was purchased directly from the manufacturer. All doses administered came from the same lot of each of the vaccines.

Inclusion criteria were as follows: age 31 to 40 years, agreeing to participate in the study by signing a term of free informed consent, absence of any serological marker of hepatitis B, and nonreagent HIV serology. Exclusion criteria included presence of immunosuppressive diseases or renal insufficiently and use of corticosteroids. In addition to these criteria, we also excluded from follow-up any volunteers who received other doses of hepatitis B vaccine in addition to those provided by the trial; missed any of the later vaccine doses or blood sample collections; or expressed the will to leave the trial at any moment.

The present study is characterized as a vaccine non-inferiority trial,[18] the goal of which is to demonstrate that the proportion of subjects with the desired immunogenic response to the novel vaccine is not inferior to that of the control group beyond a preestablished non-inferiority threshold. For sample size calculation, we used the following parameters: maximum difference in seroprotection between the two vaccines of 0.075; proportion of seroprotection in the control vaccine of 0.93; proportion of seroprotection in the experimental vaccine of 0.95; 0.95 confidence level; 90% statistical power; 20% losses to follow-up; and one-tailed statistical analysis. Using PASS statistical software to test equivalence of proportions, we determined the necessary sample size at 260 subjects per group.

The population of policemen within the age group of interest was estimated at approximately 3,000. We included 564 subjects in the sample (18.8% of the total): 283 were allocated in the experimental group and 281 in the control group. There were no differences between the groups in terms of sex, age, and other baseline characteristics. Of this total, 145 volunteers were lost to follow-up in the course of the study. Losses were distributed equally between the two groups. The major reason for loss was voluntary drop out (63.4%).

The analysis was per-protocol, including only those subjects that completed all steps of the trial: pre-vaccine serology; administration of three doses of vaccines; interval between the two first doses of 28 days or longer; interval between the second and third doses of at least 120 days; interval between the third dose and the final blood sample of 28 to 100 days; quantitative final dosage of anti-HBs.

We adopted the necessary procedures to ensure blinding, so that neither subjects nor laboratory personnel who determined anti-HBs titers had access to grouping information. Subject entries were numbered, and the vaccine to be used was randomly assigned. The group status was known by the study coordinator and fieldwork team, since vaccine presentations were different (single dose in the case of EngerixB® and 5 ml vials in the case of VrHB-IB). Adverse effect evaluation charts did not contain information on the vaccine used.

Samples were collected, centrifuged, and aliquoted by the Central Laboratory of the *Santa Casa de Misericórdia de São Paulo* (LC-SCMSP), and those from the second and third samples were sent to the

Table 1. Frequency distribution of adverse effects reported after each vaccine dose, according to type of vaccine. Municipality of São Paulo, Southeastern Brazil, 2004-2005.

Vaccine	1st dose			2nd dose			3rd dose		
	Total	With adverse effect	%	Total	With adverse effect	%	Total	With adverse effect	%
VrHB-IB	283	80	28.3	242	47	19.4	225	40	17.8[a]
Engerix®	281	87	31.0	226	37	16.4	210	22	10.5[a]
Total	564	167	29.6	468	84	17.9	435	62	14.2

[a] $\chi^2 = 4.16$, p = 0.04

Instituto Oswaldo Cruz/ Fundação Oswaldo Cruz (IOC/FIOCRUZ) for serological evaluation. The first sample was analyzed by the LC-SCMSP immediately after collection for presence of hepatitis B markers (HBsAg, total anti-HBc, anti-HBc IgM, and an-HBs) and HIV 1 and 2. Antibody titers were determined by microparticle enzyme immunoassay (MEIA) using AXSYN automated equipment (Abbot), strictly as recommended by the manufacturer. Samples reactive or inconclusive at the baseline were retested.

Quantitative dosage of anti-HBs (in international miliunits of anti-HBs per milliliter – mUI/ml) was performed on the second and third samples using a commercially available immunoenzymatic assay (ACCESS® AbHBsII, Beckman Coulter). This system is fully automatic, ensuring the reproducibility of the obtained results. For quality control purposes, 10% of samples were retested by an independent laboratory (Instituto Evandro Chagas, Ministry of Health, Belém – PA).

Seroprotection was defined as a titer equal to or greater than 10 mUI/ml. We calculated arithmetic mean, median, and geometric mean titers at each timepoint. Titers were classified into the following categories: < 10 (negative), 10 – 100 (weakly positive), and > 100 (strongly positive).

After each procedure, all data collection instruments were rechecked in search of empty fields and inconsistencies. When necessary, we contacted subjects in order to complement the available information.

Following data entry into the database, we proceeded to exploratory analysis, which consisted of calculating distribution and central tendency/dispersion to verify the success of randomization both in the original cohort and in the group of subjects that completed the protocol. For immunogenicity evaluation we considered two variables: "anti-HBs titer 3" (referent to the third blood sample) and its derivative, "seroprotection." Individuals presenting anti-HBs greater or equal to 10 UI/ml were considered seroprotected.

The proportion of adverse effects for each vaccine was compared, and differences were evaluated by chi-squared and Fisher's exact tests, adopting a significance level of 5%.

We carried out an analysis of the magnitude of losses and whether they differed between groups, which could bias the analysis.

We adopted as a criterion for significance, i.e., as a probability of the difference being significant, a p-value below 0.05.

The study protocol was approved by the Research Ethics Committee of the *Faculdade de Ciências Médicas* of the *Santa Casa de São Paulo*. The study was planned and developed independently from the manufacturers of the vaccines involved. The Term of Free Informed Consent, containing a general description of the study, its objectives, procedures, risks and benefits to the participant, guarantees in the case of occurrence of adverse effects, and the names of those responsible for the project was read and discussed jointly between a member of the team and the volunteer candidate.

RESULTS

Table 1 presents the frequency of adverse reactions reported by participants after each dose. The frequency of reported adverse reactions decreased from each dose to the next in both groups. Analyzing each dose separately, there was no significant difference in the frequency of adverse reactions to each vaccine, with the exception of the third dose, after which the incidence of adverse reactions was higher among subjects receiving VrHB-IB ($\chi^2 = 4.16$; p < 0.05). Most common complaints following immunization were pain at the site of injection, sleepiness, and headaches. No severe side effects were reported.

Table 2. Distribution of seroprotection according to vaccine type. Municipality of São Paulo, Southeastern Brazil, 2004-2005.

Vaccine	Negative		Positive		Total	
	n	%	n	%	n	%
VrHB-IB	3	1.4	213	98.6	216	100.0
Engerix®	9	4.4	194	95.6	203	100.0
Total	12	2.9	407	97.1	419	100.0

$\chi^2 = 2.48$, p = 0.11

Table 3. Intensity of seroprotection according to vaccine type. Municipality of São Paulo, Southeastern Brazil, 2004-2005.

Vaccine/Seroconversion	Strong		Weak		Negative		Total	
	n	%	n	%	n	%	n	%
VrHB-IB	206	95.4	7	2.2	3	1.4	216	100.0
Engerix®	172	84.7	22	10.8	9	4.4	203	100.0
Total	378	90.2	29	6.9	12	2.9	419	100.0

Seroprotection was assessed in the samples collected one month after the third dose of vaccine. Table 2 shows that only 12 of the 419 samples tested were negative, i.e., only 12 subjects (2.9% of the total) were not seroprotected after having received three doses of the vaccine. This proportion was 1.4% and 4.4% for VrHB-IB and Engerix B®, respectively. The difference between the vaccines was not significant. The geometric mean titers for each vaccine were 12,577 and 11,673, respectively, for the IB vaccine and for Engerix B®. The Kruskal-Wallis statistic for this comparison was 5.04, i.e., mean antibody titers obtained with the the IB vaccine were significantly higher than those obtained using Engerix B®.

Table 3 shows that 90.2% of vaccinated subjects were strongly reactive. This included 95.4% of subjects in the IB vaccine group and 84.7% of those in the Engerix B® group, a statistically significant difference ($\chi^2 = 13.4$; $p < 0.01$).

For quality control purposes, the anti-HBs titers of 61 randomly selected samples were retested by a different laboratory. The intraclass correlation coefficient between the two tests was 0.973 (95%CI: 0.955;0.984), evidence of a strong correlation.

DISCUSSION

The hepatitis B vaccine manufactured by Instituto Butantan showed a similar safety profile to that of the reference vaccine. Most adverse events reported consisted of localized reactions and non-specific symptoms. As reported for other recombinant hepatitis B vaccines, we detected no severe adverse effects.[18]

In a prior trial[17] in volunteers of the same age group, a significant difference was detected in the immunogenicity of the IB vaccine when compared to the reference, exceeding the previously established limit of 5%. In the group that received the IB vaccine (20 µg), 79.8% of subjects were effectively immunized, vs. 92.4% of those receiving the reference vaccine.[17] In the present trial, using the 25 µg dose, the immunogenicity of the

IB vaccine was virtually identical to that of the standard vaccine, with high antibody titers being induced in the majority of subjects (95.4% of titers higher than 100mUI/ml).

A range of factors may influence the results of trials of recombinant hepatitis B vaccines, such as, for instance, differences in formulation and manufacturing, vaccination schedule, age at vaccination, site of vaccine administration, the laboratory test used to determine results, and differences between study populations, among others. Thus, comparisons between different studies should be undertaken with caution. On the other hand, proper randomization of volunteers into experimental and control groups ensures that factors potentially associated with the results are uniformly distributed. In the present study, we detected no difference between groups regarding baseline characteristics, showing that randomization was successful.

The proportion of losses was somewhat higher than expected. Considering that they were distributed equally between the two groups, and also given the robustness of our results, we consider that such a proportion of losses would be unlikely to influence the results of the trial.

Both vaccines achieved strong seroconversion (above 100 U of anti-HBs per ml) after the third dose, with high mean titers. The results obtained show that the two vaccines are equivalent in terms of immunogenicity and reactogenicity among the 31-40-year-old population, and therefore that the Instituto Butantan vaccine can be widely used for the control of such an important endemic disease in Brazil.

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