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Efficacy and safety of the Brazilian vaccine against Hepatitis B in newborns

ABSTRACT

OBJECTIVE: To analyze the efficacy and safety of a recombinant Hepatitis B vaccine in newborns.

METHODS: The study was carried out in a general hospital in the city of Guarulhos, Southeastern Brazil, between 2002 and 2005. The recombinant Hepatitis B vaccine from Instituto Butantan (VrHB-IB) was tested in two clinical trials. In both trials, newborns were randomly allocated to the experimental or control (reference vaccine) groups. Newborns were given three doses of vaccine, one up to 24 hours after birth and the other two 30 and 180 days later. In the first trial, 538 newborns completed the immunization protocol, and 486 in the second. Vaccines were considered equivalent when seroprotection difference was below 5%.

RESULTS: Seroprotection in the first trial (anti-HBs ≥ 10mUI/ml) was 92.5% (247/267) in the experimental group, compared to 98.5% (267/271) in the control (p = 0.001). With this result, VrHB-IB did not fulfill the pre-established criterion for equivalence. After increasing the concentration of antigen in the vaccine to 25µg, seroprotection reached 100% in the experimental group and 99.2% in the control. No severe adverse effects were recorded.

CONCLUSIONS: The reformulated VrHB-IB is considered equivalent to the reference vaccine, and its use is recommended in newborns.

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

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INTRODUCTION

Infection by the hepatitis B virus is an important public health problem in Brazil. Certain regions in the country are regarded as hyperendemic, including the Western Amazon as well as several microregions in the states of Espírito Santo, Paraná, Santa Catarina, and Mato Grosso. After the introduction of universal vaccination, there has been an important reduction in prevalence of chronic infection in a number of these areas.[3]

Since the late 1980's, all available vaccines are produced using the techniques of molecular biology, in which vaccine antigen is produced using recombinant DNA technology.

The recombinant vaccine against hepatitis B is highly immunogenic and protective. A response is considered to be protective when the vaccine is able to induce the formation of antibodies to HBsAg (anti-HBs) at levels ≥10 mUI/ml as measured by immunoenzymatic assay. A complete series of three or four doses of hepatitis B vaccine is capable of inducing a protective response in over 90% of healthy adults and over 95% of healthy children and adolescents. Most vaccination regimens recommend three or four doses of vaccine (at zero, one, and six months or at zero, one, two, and 12 months, respectively). The first doses induce detectable antibodies to HBsAg in approximately 70% to 85% of vaccinees, but antibody levels are relatively low (50-300 mUI/ml). the final dose induces an adequate response in around 90% of adults and in over 95% of children, antibody levels increasing by 1,000 – 3,000 mUI in adults and usually by more than 5,000 mUI/ml in children.[4]

Studies conducted in the United States have demonstrated the safety of the hepatitis B vaccine based on an evaluation of 12 million doses given to infants. Side-effects are similar between all licensed hepatitis B vaccines. Pain and hyperemia at the injection site are the most frequent adverse effects (15%-20%), and are probably related to the vaccine adjuvant – aluminum hydroxide. Approximately 15% of vaccinees experience one or more mild, self-limiting, systemic symptoms, including cephalea, fever, and/or fatigue, usually 24 to 48 hours after inoculation.[2,10]

The Brazilian Ministry of Health's National Immunization Program began to incorporate hepatitis B vaccination in 1992, in vaccination campaigns taken place in the country's hyperendemic areas. In 1998, the recombinant hepatitis B vaccine was incorporated into the universal vaccination program for infants in the entire country.

The Instituto Butantan in São Paulo has developed a hepatitis B vaccine (VrHB-IB) produced using recombinant DNA. The development of a molecular biology-based vaccine constitutes a further step toward towards self-sufficiency in the production of immunobiologicals in Brazil, which reduces both our reliance on imports and the price of the vaccine, in addition to ensuring vaccine supply for the universal program.

The VrHB-IB vaccine contains highly purified particles of HBsAg produced in recombinant yeast (*Hansenula polymorpha*), and its formulation includes the adjuvant aluminum hydroxide with initially 20µg, and later 25µg, of recombinant antigen per ml of diluent. Preliminary studies using 10µg per dose in a zero, six, and nine-month regimen, administered to healthy adult voluntaries, showed that VrHB-IB did not induce significant adverse effects, with seroconversion reaching 95.3%.[1] This vaccine was later found to induce a weaker immunogenic response in subjects older than 45 years, where seroconversion was 70%, compared to 100% in the 18-25 years age group. Furthermore, differences in geometric mean titers induced by 10µg and 20µg doses led the authors to consider the need for increasing antigen concentration in the vaccine.[5] VrHB-IB was licensed for use in Brazil in 1998, and has been in wide use by National Immunization Program since 2003.

Subsequent trials have shown VrHB-IB to be equivalent in efficacy to the reference vaccine in children from one to 11 years of age, "less immunogenic, but acceptable for use in newborns, adolescents, and young adults," and significantly less immunogenic in adults aged 31 to 40 years.[6] In light of these results, the manufacturer has increased the concentration of antigen in the vaccine from 20 to 25µg/ml. This has generated the need for further efficacy trials for the vaccine, not only focusing on the group in which the difference in immunogenicity was greatest – adults – but also on newborns, the target group of National Immunization Program.

The objective of the present study was to analyze the efficacy and safety in newborns of this recombinant hepatitis B vaccine.

METHODS

The safety and efficacy in newborns of the hepatitis B vaccine produced by Instituto Butantan (VrHB-IB) were evaluated in two randomized, double-blind clinical trials.

Newborns participating in each of the trials were given three 0.5 ml doses of hepatitis B vaccine by intramuscular injection. The first dose was given up to 24 hours after birth, the second, 30 days later, and the third, 180 days after the first. Newborns randomized to the experimental group received VrHB-IB, and those in the control group received the reference vaccine, a Engerix B®, produced by Glaxo Smith Kline. Both vaccines

used in the first trial contained 20μg/ml of antigen. In the second trial, we used a new formulation of VrHB-IB containing 25μg/ml of antigen. In both trials, a single lot of the experimental and control vaccines was used.

The first trial was carried out in 2002-2003, and the second in 2004-2005. All newborns delivered at a general hospital in the city of Guarulhos, Southeastern Brazil, were eligible for participation. We excluded babies born to mothers who were HBsAg carriers or with positive serology for HIV or syphilis; born before term (gestational age < 37 weeks); with low birthweight (<2,000 g); carrying congenital malformations, genetic diseases or other severe clinical conditions; with 1 minute Apgar < 7 or 5 minute Apgar < 8; and who received exchange transfusions or intravenous immunoglobulin.

Expecting parents were approached by the study team upon admission to the hospital for delivery. During this initial interview, parents were introduced to the study and invited to participate. In case of acceptance, a further interview was conducted after delivery, when a Term of Free Informed Consent was read to and discussed with parents. Those accepting to participate in the trial signed the Term, which was also signed by a representative of the study team. Newborns included in the study were vaccinated within the first 24 hours of life, and a return appointment was scheduled. The study team offered to follow-up enrolled newborns throughout the first year of life.

Upon recruitment, each newborn was assigned a sequential number corresponding to the order of enrollment in the study. Sequential numbers had been previously randomized, with an independent probability of 50% for each number. Only the professional responsible for the randomization and allocation of sequential numbers to each of the groups had access to this information. None of the professionals responsible for subject follow-up or for the laboratory testing had access to the randomization information. Vaccine vials were modified so as to prevent their identification.

Equivalence trials are conceived of as trials of noninferiority of the tested vaccine.[10] In such trials, one attempts to show that the proportion of subjects with the desired immune response after receiving the new vaccine is not lower than that of the reference group by more than a prestated margin of noninferiority. In the two trials, we accepted as indicative of noninferiority differences smaller than 5%. Using as a reference the immunogenicity of the control vaccine in Brazil (98.5%),[8] we calculated the necessary sample to detect a difference of up to 5%, with a significance level of 0.05 and 80% statistical power. This yielded a sample size of 258 subjects in each group. After adding 20% to compensate for potential losses, we arrived at a total of 610 subjects, divided equally between experimental and control groups.

The serological marker of vaccine efficacy (outcome variable) was the detection of anti-HBs antibodies in levels equal to or greater than 10 mUI/ml. Serology was performed on samples collected at T 180 (five months after the second dose of vaccine, immediately prior to the third dose), and T210 (one month after the third dose). In addition, we collected blood from the mother at the time of delivery to determine eligibility. Samples were collected, centrifuged, and aliquoted by the investigation unit and processed by the laboratory of the study hospital. Samples from newborn mothers were analyzed for serological markers of hepatitis B (HBsAg and anti-HBc), HIV, and syphilis. The second and third samples of all subjects were analyzed for serological markers of hepatitis B (HBsAg, anti-HBs, and anti-HBc). Antibody levels were measured by immunoenzymatic assay (ELISA) using commercially available reagents DiaSorin and Access (Access® AbHBsII, Beckman Coulter), the latter for measuring anti-HBs. In the first trial, anti-HBs testing was performed by Instituto Adolfo Lutz (IAL) and Instituto Evandro Chagas (IEC), and in the second trial, by Instituto Oswaldo Cruz. In the first trial, all laboratory tests were carried out simultaneously by the participating laboratories (IAL and IEC) using different commercially available reagents (DiaSorin at IAL and Access at IEC). In the second trial, a subsample of 65 volunteers was retested at IEC.

To evaluate the vaccine's safety, we recorded any adverse effects attributable to the vaccine after the day of vaccination. Adverse effects were actively monitored during the first 72 hours after vaccination, by visiting mother and newborn in the hospital after the first dose, and by telephone after the second and third doses. After 72 hours, monitoring was passive, relying on spontaneous reporting by the mother or care giver and on inquiry upon the subject's return to the hospital for neonatal care and vaccination.

We included in the analysis only newborns who completed all stages of the follow-up protocol. Losses were distributed equally between experimental and control groups in both trials (Table 1). In the first trial, 86.4% of the 630 newborns completed all stages of the protocol. In the second trial, 82.2% of the 590 randomized newborns completed follow-up. Major reasons for loss to follow-up were failure of the subject to show up for a scheduled appointment and inability of the research team to locate the family at the given address; hepatitis B vaccination somewhere other than the trial unit; and dropout from the trial by request of the family. We found no differences between losses and newborns that completed all follow-up stages in terms of any of the variables investigated.

We compared the proportion of seroprotection and geometric mean anti-HBs titers between experimental and control groups. We calculated the difference in

Table 1. Distribution of newborns participating in the trial according to selected variables at the time of recruitment. Guarulhos, Southeastern Brazil, 2002-2005.

Parameter	First trial		Second trial	
	Experiment group (n = 315)	Control group (n = 315)	Experimental group (n = 298)	Control group (n = 292)
% males	52	52	51	50
Mean weight (g)	3.251	3.216	3.232	3.282
Mean 5′ Apgar	9.36	9.35	9.35	9.37
Mean length (cm)	48.5	48.5	48.8	49.0

proportions and their 95% confidence intervals.[10,11] We carried out univariate analysis of cofactors that could potentially influence the outcome. We also compared the frequency of adverse events between the two groups after each dose of vaccine. For data analysis, we used Epi Info and SPSS 13.0 software.

The study was approved by the Research Ethics Committee of the Irmandade de Santa Casa de Misericórdia de São Paulo (1st trial: Process no. 061/01; 2nd trial: Process no. 221/03).

RESULTS

Table 2 presents the distribution of subjects in the experimental and control groups according to selected variables at the time of enrolment. In both trials, groups were equally distributed in terms of the investigated variables with no significant differences between groups, which indicates successful randomization.

In the first trial, the difference in efficacy between VrHB-IB and the reference vaccine was 6% (Table 3), i.e., higher than the prestated equivalence margin of 5%. However, this value was within the 95% confidence interval of the difference in proportions. In addition to this variable, no other covariable was significantly associated with the outcome. In the second trial, there was no difference in efficacy between the two vaccines (Table 4). As in the first trial, there was no association between the outcome variable and any of the covariables investigated.

Also in the first trial, geometric mean antibody levels in the experimental and control groups were 420.8 and 1,769.9 mUI/ml, respectively. In the second trial, mean levels were 2,616 and 10,051 mUI/ml, respectively. These differences were significant in both trials. In the first trial, the intraclass correlation coefficient between the results of serological tests (antibody levels) from the two laboratories using distinct commercial kits was 0.878 (95% CI: 0.856; 0.897). In the second trial, the intraclass correlation coefficient between the two labs, using the same commercial kit, was 0.992 (95% CI: 0.959; 0.997).

Regarding reactogenicity, families reported an increase in adverse effects between the first and third doses in both trials. Localized reactions were the most frequently reported events after the first dose. After the second and third doses, most reports were of increased crying/irritability and low fever. Almost all adverse

Table 2. Distribution of losses to follow-up according to study group. Guarulhos, Southeastern Brazil, 2002-2005.

Group	First trial		Second trial	
	Losses	n	Losses	n
Experimental	50	315	56	298
Control	47	315	49	292
Total	97	630	105	590

Table 3. Results of anti-HBs serology after the third dose of vaccine in the first trial. Guarulhos, Southeastern Brazil, 2002-2005.

Vaccine	Anti-HBs serology		Total	% seroprotection
	Non-reactive	Reactive		
Experimental	20	247	267	92.5
Control	4	267	271	98.5
Total	24	514	538	95.5

χ^2 (Yates correction) = 10.48 p = 0.001
D = - 6 (95% CI: –9.5;–2.5).
D: Difference in proportions.

Table 4. Results of anti-HBs serology after the third dose of vaccine in the second trial. Guarulhos, Southeastern Brazil, 2002-2005.

Vaccine	Anti-HBs serology		Total	% seroprotection
	Non-reactive	Reactive		
Experimental	-	242	242	100.0
Control	2	241	243	99.2
Total	2	483	485	99.6

χ^2 (Yates correction) = 0.50 p = 0.48
D = 0.8 (95% CI: 1.9;–0.3).
D: Difference in proportions.

Table 5. Distribution of the frequency of adverse effects after vaccination against hepatitis B, according to dose and vaccine used. Guarulhos, Southeastern Brazil, 2002-2005.

Dose	First trial		Second trial	
	Experimental n	Control n	Experimental n	Control n
First	6	10	2	4
Second	6	14	30	42
Third	47	22*	45	41

* p < 0,01

events were observed in the first 72 hours after vaccine administration. The third dose was administered simultaneously with the DTP vaccine. No severe adverse reactions were observed in any of the trials. Only after the third dose in the first trial was the frequency of adverse events significantly higher in one of the groups (VrHB-IB). No differences in frequency of adverse effects between the two vaccines were detected after the first two doses in the first trial, and after all doses in the second trial (Table 5).

DISCUSSION

After increasing antigen concentration to 25μg/ml, the immunogenicity of VrHB-IB was practically identical to that of the reference vaccine, having induced high antibody titers in the majority of volunteers (97.5% with titers higher than 100 mUI/ml). In the two trials, the null hypothesis of noninferiority could not be rejected.

VrHB-IB showed a similar reactogenicity and safety profile to that of the reference vaccine. The majority of adverse effects recorded consisted of localized reactions and low fever. Only at one of the analyzed time points – after the third dose of the first trial – we observed a significant difference in the frequency of adverse effects between the two vaccines. Considering that these were mild events, and that this excess of adverse events did not repeat itself in the second trial, it is possible that such difference was due to chance. The administration of DTP vaccine concomitantly with the third dose of hepatitis B vaccine may have led to the greater frequency of adverse effects reported after this dose, but not for the difference observed between the two vaccines. As in other trials assessing the safety of other recombinant hepatitis B vaccines, no severe adverse effects were reported.[7]

Several factors can influence the results of recombinant hepatitis B vaccine trials, including differences in vaccine formulation and production, different vaccination regimens, age at vaccination, site of vaccine administration, concomitant immunization with other vaccines, differences in the laboratory assays used for outcome measurement, and differences between the study populations, among others. Therefore, comparisons between different studies should be undertaken with caution.

Our present results describe part of the course of development of VrHB-IB. The first trial, combined with the results reported by Martins et al,[6] led to an increase in concentration of antigen in the vaccine, and the results of the following trial confirmed the expected increase in immunogenicity of the reformulated product. Our present results in newborns, along with those of another trial conducted simultaneously, by the same team, in a sample of adults, allow us to conclude that the performance of VrHB-IB is identical to that of the reference vaccine, warranting its large-scale use in the control of an important endemic disease of in Brazil.[9]

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