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SAFETY AND IMMUNOGENICITY OF HEPATITIS B VACCINE BUTANG IN ADULTS

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SUMMARY

Recombinant yeast-derived hepatitis B vaccine manufactured by Instituto Butantan was administered in two groups of adult volunteers (I, II) following two different schedules of immunization. In the first trial (10 µg doses and 0, 1, 3 months vaccination schedule) 106 individuals completed the full immunization program. The results of seroconversion by age group varied from 70 to 100% and the GMT from 46.5 to 124.9 mIU mL^{-1}. In the second trial with 68 individuals (for dosage comparison and 0, 1, 6 months vaccination schedule) indicated that the vaccine formulated in 20 µg was more effective than in 10 µg. The adverse reactions observed in the vaccinees were less frequent than the ones previously found since the introduction of similar vaccines.

KEYWORDS: Hepatitis B vaccine; Clinical trials.

INTRODUCTION

Hepatitis B is an extremely serious disease parenterally/sexually transmitted, representing the 9th cause of morbidity and mortality in the planet. The infection with hepatitis B virus (HBV) leads to a variety of clinical manifestations: fulminant, acute, chronic and inapparent. Fulminant and acute forms are severe and cause of high mortality. Chronic hepatitis is the form responsible for virus spreading and can potentially progress to cirrhosis and liver cancer. When the infection occurs in early age (newborns to 1 year) the evolution to a chronic state is about 90%, decreasing with the age[4]. More than one million chronic carriers die every year[13]. The HBV is spread in Brazil in regions of low, medium and high prevalence: in the South the incidence is about 0.3 to 1.7%, in São Paulo and Rio de Janeiro from 1.0 to 2.1% and in the Amazon region from 2.8 to 10.3% of the population[3].

Prevention by vaccination is the only effective strategy to avoid the disease. Since 1987 two licensed yeast derived recombinant vaccines containing the major hepatitis virus surface protein (S-protein, HBsAg) were available, manufactured by Merck Sharp Dohme (USA) and Smith Kline Beecham (Belgium) and they are still leading the market of HB vaccine[6]. Evaluation made by FDA (Food and Drug Administration) recognized the safety of hepatitis B vaccine based on 12 millions doses administered to babies up to 12 months age[8]. Tiredness, erythema and soreness in the local of injection are the most common side effects observed mostly concerned to the aluminum hydroxide present in formulated product as vaccine adjuvant. The Advisory Committee on Immunization Practice of the Centers for Disease Control and Prevention (CDC) and the American Academy of Pediatrics recommended the vaccination as the only practical means to reduce the HBV incidence in the USA[9]. In China there was a drastic reduction of 60% of new cases due to the application of an immunization program for newborns[10].

The Instituto Butantan in São Paulo (Brazil) produces a recombinant yeast-derived Hepatitis B vaccine (ButaNG) by technology developed in cooperation with N.G. Biotecnologia Ltda. The vaccine is manufactured and quality controlled following the requirements established by World Health Organization and belongs to the new generation of safe vaccines. The vaccine contains highly purified recombinant HBsAg particles produced in yeast *Hansenula polymorpha* and formulated in aluminum hydroxide. Initially ButaNG was formulated in adult dose containing 10 µg of HBsAg, based on results of mice immunization experiments utilizing simultaneously Engerix B vaccine as a reference. Engerix was selected due to its well known efficacy. The vaccination followed the schedules approved by FDA of 0, 1, 6 and 0, 1, 3 and 12 months.

The Instituto Butantan is ready to supply the national vaccine demand for a mass immunization program estimated to be around 30 millions annual doses.

MATERIALS AND METHODS

Vaccines: ButaNG lots: 9709141 (10 µg protein, 0.25 mg Al^{+++}/dose); 9710151 (10 µg protein, 0.25 mg Al^{+++}/dose); 9710154 (20 µg protein, 0.25 mg Al^{+++}/dose)

Engerix B lots: 2202A4, 2243A4 and 2272B4 (20 µg protein, 0.50 mg Al^{+++}/dose)

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ButaNG vaccine was initially formulated in aluminum hydroxide in doses of 10 µg/mL based on the comparative results obtained when ButaNG and Engerix B (used as reference vaccine) were injected in BALB/c mice. Doses of 0.5 µg of protein per animal were inoculated in groups of 20 mice, in a single shot. Blood samples collected 30 days after inoculations were analyzed by ELISA kits (AUSAB-EIA, Abbott Lab.). The results are presented in Table 1.

Subjects

The volunteers (18-57 years) were recruited from the Hospital's staff of Fundação Amaral Carvalho, Jaú-SP. The criteria for selection was restricted to the absence of any serological HBV markers assayed by Abbott Lab. kits (Corzyme, Auszyme, Ausab) or to serious health problems.

The reactogenicity was observed after each dose when the patients were inquired for abnormalities such as: injection site pain, headache, fever, fatigue, influenza like symptoms, diarrhea and others.

Study design

The first trial with group I started with 180 individuals aged 18-57 years. They were injected intramuscularly in the deltoid region with 10 µg of ButaNG lot 9709141, amount of protein estimated by the results obtained with mice experiments (Table 1). The schedule of immunization was 0, 1, 3 months.

Based on the results of immunogenicity obtained for group I of vaccinees, a second trial in group II of 68 individuals aged 18-50 years was initiated. This group was homogeneously subdivided in: A, B, C, D subgroups, considering the age, sex and factor weight/height and vaccinated with ButaNG 9709141, 9710151, 9710154 and Engerix B 2243A4, respectively. They were injected in the deltoid muscle following the normal recommended schedule of 0, 1, 6 months.

Table 1
Comparative potency of different lots of ButaNG and reference vaccine, in BALB/c mice

Vaccine lot#	Seroconversion (%)	Anti-HBs GMT (mIU mL^{-1})
EngerixB	80	63.7
2202A4	25	142.2
	100	151.2
EngerixB	93	239.6
2243A4	70	138.7
	80	151.8
EngerixB	93	84.0
2272B4	100	124.9
	10	26.5
ButaNG	75	158.3
9709141	75	125.4
ButaNG	100	310.8
9709151	92	152.9
	100	142.4
ButaNG	100	264.0
9710154	100	130.0
	100	117.7

Blood specimens were collected from volunteers before the first injection and evaluated to be negative for the presence of hepatitis B surface antigen (HBsAg) and antibodies to core antigen (anti-HBc) and to HBsAg (anti-HBs). Samples collected from vaccinees one month after receiving the third shot were tested for anti-HBs. Antibodies were measured in GMT (geometric mean titer) expressed in mIU mL^{-1}. All blood specimens were tested following the instructions of enzyme-linked immunosorbent assay kit (AUSAB-EIA, Abbott Lab.).

Seroconversion was defined as antibody GMT $\geq$ 2.1 mIU mL^{-1} and seroprotection as $\geq$ 10 mIU mL^{-1}.

RESULTS AND DISCUSSION

Safety

Short term adverse reaction data reported by individuals from group I after receiving the first shot were injection site soreness (21.7%) sometimes accompanied by erythema, fatigue or headache (4.3%) and low fever (1.6%). Reactions were less frequent with subsequent doses. The symptoms, mainly the arm soreness were less frequent than the ones reported regarding recombinant hepatitis B vaccines[1,12]. Adverse reactions reported by group II were basically arm soreness in 30% of vaccinees after receiving the first dose of Engerix B and in 20% of individuals who received ButaNG. The less frequent reactions observed can be attributed to the low concentration of aluminum present in the formulation of ButaNG.

The immune response to ButaNG 9709141 in the 106 individuals of group I is shown in Table 2 divided by age groups. The seroconversion decreased with age and the GMT was significantly lower after the age 35. A lower anti-HBs GMT than those found for similar vaccines was expected due to the short schedule of immunization utilized[2,5,7]. Previous published data showed that intervals of 5 to 10 months between the second and third doses maximized the antibody response[11]. In this

Table 2
Immune response to ButaNG vaccine in pediatric dose (10 µg) in group I of 106 vaccinees separated by age group

Age (years)	number	seroconversion (%)	anti-HBs GMT* (mIU mL^{-1})	≥10 mIU mL^{-1} (%)
18-25	21	100	124.9	86
25-34	42	88	140.9	80.5
35-44	33	85	55.9	78.5
45-57	10	70	46.5	100

*Serum anti-HBs levels measured with ELISA kits (Ausab EIA-Abbott Lab.).
in GMT (geometric mean titer).
10 mIU mL^{-1}, protective antibody level.

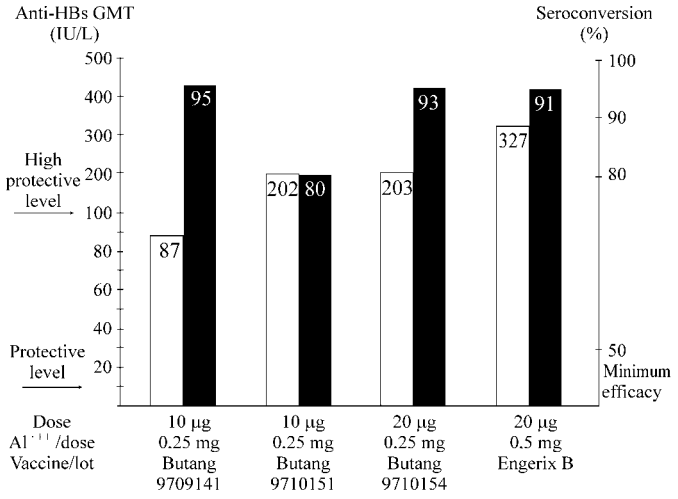

Fig. 1 - Comparative potency in group II of different lots and doses in terms of anti-HBs GMT (□) and seroconversion (■).

group the highest titer was 1597 mIU mL^{-1}. For the seronegative individuals or to those who showed GMT under 10 mIU mL^{-1} (protective level) a booster in the 12 month was recommended.

As the immune response to 10 µg of HBsAg did not confirm the enhanced immunogenicity observed in mice experiments (Table 1), it directed us to use a second study design in order to determine some suspected reasons: lower concentration of antigen and aluminum than those used in the formulation of similar vaccines and shorter schedule of immunization usually applied to individuals under risk of infection.

The 68 subjects aged 18-50 were distributed in subgroups A, B, C, D and vaccinated with different vaccine lots, doses and with reference vaccine by the schedule of 0, 1 and 6 months. Figure 1 shows the comparative potency of vaccines ButaNG formulated in doses of 10 and 20 µg with Engerix B in dose of 20 µg. The difference between the GMTs determined for 10 and 20 µg doses shows that the antigen concentration in the vaccine has to be increased and tested in bigger population. The highest titer detected in mIU mL^{-1}/the standard deviation (std.dv.) for subgroups A, B, C and D were 540/141, 646/244, 560/190 and 597/191, respectively.

The clinical trials with ButaNG vaccine was considered satisfactory in terms of low rate of reactogenicity, absence of any incapacitating side effects, seroconversion equivalent to similar vaccines and induction of protective levels of antibodies. The response to 20 µg ButaNG (GMT 203 mIU mL^{-1}) is superior than the titer considered as high protective level (100 mIU mL^{-1}).

The vaccine has the potency to have its efficacy improved by optimizing the formulation by the introduction of different adjuvants or simply by adjusting the concentration of all vaccine components.

RESUMO

Inocuidade e imunogenicidade da vacina contra a hepatite B, ButaNG, em adultos

Vacina contra a hepatite B, produzida no Instituto Butantan em levedura recombinante, foi administrada em dois grupos de voluntários adultos (I e II) seguindo dois esquemas diferentes de imunização. No primeiro ensaio (doses de 10 µg e esquema de vacinação de 0, 1, 3 meses), 106 indivíduos completaram o programa de imunização proposto. Os resultados de soroconversão agrupados por faixa etária variaram de 70 a 100%, enquanto que o TGM foi de 46,5 a 124 mUI mL^{-1}. No segundo ensaio com 68 indivíduos (comparação de doses e esquema de vacinação de 0, 1, 6 meses) os resultados indicaram que a vacina formulada em dose de 20 µg foi mais eficaz que em dose de 10 µg. Os efeitos adversos observados nos vacinados foram menores do que os relatados por outros autores, desde o início da aplicação de vacinas similares.

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