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• *BRIEF REPORTS* •

Immune response to an indigenously developed r-Hepatitis B vaccine in mixed population: Study of an accelerated vaccination schedule

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Abstract

AIM: To establish the safety and efficacy of an indigenously developed r-hepatitis B vaccine using an accelerated schedule and to highlight the social awareness and commitment in preventing the spreading of hepatitis B virus infection.

METHODS: The study was a multicentric, double blind, randomized (3:1) study using three doses of vaccine immunization schedule (20 μg for those above 10 years old and 10 μg for those below 10 years old) on d 0, 30 and 60. One hundred and sixty-six subjects were enrolled (87 males and 76 females aged 5-35 years). The main outcome measure was assessment of immunogenicity and safety.

RESULTS: A 100% seroconversion response was observed on the 30th d after the 1st injection in both the experimental groups. The sero-protection data reported a 41.2-65.6% response on the 30th d after the 1st injection and reached 100% on the 60th d. Descriptive statistical analysis showed a geometric mean titer value of 13.77 mIU/mL in the test (BEVAC) group and 10.95 mIU/mL in the commercial control (ENGERIX-B) group on the 30th d after the 1st injection. The response on the 60th d showed a geometric mean titre value (GMT) of 519.84 mIU/mL in the BEVAC group and 475.46 mIU/mL in the ENGERIX-B group. On the 90th d, the antibody titer response was observed to be 2627.58 mIU/mL in the BEVAC group and 2272.72 mIU/mL in the ENGERIX-B group. Two subjects in each group experienced pains at injection site after the first vaccination. A total of six subjects in both groups experienced a solicited adverse reaction, which included pains, swelling and redness at the injection site, three subjects in the group-B had a pain at the injection site after the third dose. No other serious adverse events occurred and no dose-related local or general symptoms were observed during the study.

CONCLUSION: The vaccine is safe, efficacious and immunogenic in comparison with the well documented ENGERIX-B.

Key words: Hepatitis B; r-Hepatitis B Vaccines; Immune response; Accelerated vaccination schedule

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INTRODUCTION

For over two centuries, active immunotherapeutic approaches have been at the forefront of efforts to prevent the infectious diseases that plague humans. In the 18th century in Europe, smallpox caused 10% of all deaths. Edward Jenner's remarkable achievements in 1796 with smallpox vaccination has opened up new vistas for the development of prophylactic vaccination against the present day killer infectious diseases, including typhoid, cholera, plague, measles, hepatitis-B. Out of these dreaded infectious diseases, worldwide prevention of chronic HBV infection has become an ultimate priority.

Viral Hepatitis is a disease with multiple causes and a public health problem, which was first described in the 5th century BC by Hippocrates[1]. Hepatitis-B virus infection is a common viral disease and the present data show that more than one third of the world population are infected with this virus and nearly one million deaths occur every year due to this infection[2]. Epidemiological data also reveal that there are 360 million carriers of hepatitis-B virus throughout the globe and 78% of the world population are living in Asia[3].

Although safe and effective vaccinations have been available since 1980 s, universal vaccination is still postponed in many countries[4]. The reason behind inhibition or weakness of our social commitment to preventive vaccines is lack of public awareness. In India the availability of prophylactic hepatitis-B vaccines was started simultaneously by multi national companies (MNCs) and after that many others have been licensed, claimed to be efficacious and safe. But the usage is very poor due to prohibitive cost and lack of knowledge of identifiable risk factors due to weakness of

our social commitment and to preventive medicines and vaccines[5]. The present study was carried out not only to prove the safety and efficacy of an indigenously developed hepatitis-B vaccine in an accelerated schedule, but also to highlight the social awareness and commitment, in preventing the spreading of hepatitis-B virus infection.

MATERIALS AND METHODS

Vaccines

The experimental vaccine was a second-generation of recombinant vaccine derived from yeast - *Pichia pastoris*. One mL of vaccine contained 20 μg of purified hepatitis-B surface antigen, 0.5 mg % aluminum hydroxide as adjuvant (as Al^{+++}) and 0.05 mg % of thiomersal as preservative (BEVAC- HBM01002, 01102, DOM- Nov 02, DOE - Oct 05). For comparative purpose, a commercially available vaccine (ENG3449B2, DOM - Mar-02, DOE - Feb 05, ENG5322A4, DOM - Apr 02, DOE - Mar 05) manufactured by Glaxo-Smith Kline with a similar composition was used.

Study design

The study design was approved by the Institutional Ethics Committee of selected trial centers in accordance with the Declaration of Helsinki, 1989. The study was conducted after the necessary approval was obtained from the Office of the Drug Controller General of India. The study was a multi centric, double blind, randomized (3:1) study using three doses of vaccine immunization schedule (20 μg for those above 10 years old and 10 μg for those below 10 years old) on d 0, 30 and 60. Subjects were allocated randomly in two groups to receive either BEVAC or ENGERIX-B. The sample size calculation was assumed to be equivalent.

For enrollment, 200 subjects were screened at first, 34 subjects were excluded and only 166 were enrolled for the study. The subjects were randomly divided into two groups in 3:1 ratio (BEVAC - 3: ENGERIX-B - 1). All the 166 subjects received the first vaccination during their second visit. The standard flowchart is provided in Table 1.

Serological analysis

Blood samples collected during different visits were subjected to serum separation and preserved at a proper temperature. Hematology, liver function test (LFT), kidney function test (KFT) were carried out within two hours after serum separation. Different hepatitis markers were also analyzed as mentioned in the standard flowchart. All the serological parameters were carried out by the commercially available kit.

Table 1 Flowchart of the events

CRITERIA	d -7	d 0	d 30	d 60	d 90
Informed consent		v			
Medical history	v				
Physical exam/signs and symptoms	v	v	v	v	
Hepatitis B vaccine		v	v	v	
Adverse experiences		v	v	v	v
Specialty tests-HIV	v				
Hematology & ESR	v				v
Liver function test	v				v
Renal function test	v				v
HBsAg	v				
Anti-HBs Ab	v		v	v	v
Anti-HBc – IgM	v				

The seroconversion rate and geometric mean titer were measured to evaluate the immunogenicity in each group at all time points at which blood samples were taken. Seroconversion was defined as the presence of hepatitis B surface antibody titre ⩾1 mIU/mL, while antibody titer ⩾20 mIU/mL was considered sero-protective. Anti-HBs antibody was determined by using the EIA kit manufactured by Abbott Laboratories, USA (AUSAB).

Data analysis

Data analysis was carried out by the CRA Group of Biological E Limited using SPSS version 11.0.0 and Microsoft Excel 2002. The proportions of sero-converted and sero-protected subjects at different time points were studied by logistic regression. Hematological, renal function and liver function parameters analysed at different time points, were studied by "Student's *t* test" analysis. Geometric mean titer with confidence interval for each study group was also assayed and one way ANOVA was also carried out for the assessment.

RESULTS

One hundred and sixty-six subjects were enrolled for the study, data from 3 subjects were discarded, 2 for loss of essential data and 1 for non-compliance with the protocol. These subjects were randomly allocated into group A (BEVAC) and group B (ENGERIX - B). In group A, 62 males and 59 females aged 5-35 years were enrolled. Group-B consisted of 25 males and 17 females with their age similar to group A.

The data on immunogenicity showed that both the study drugs, BEVAC and ENGERIX-B were highly immunogenic (Table 2). The percent of seroconversion data depicted an

Table 2 Immunogenicity analysis in subjects after three doses of vaccination

Groups	30th d					60th d					90th d				
	GMT	AMT (mean ± SE)	Confidence Level (95%)		% SC	GMT	AMT (mean ± SE)	Confidence Level (95%)		% SC	GMT	AMT (mean ± SE)	Confidence Level (95%)		% SC
			U	L				U	L				U	L	
BEVAC	13.77	33.30± 9.86	52.83	13.76	100	519.84	632.67± 3.94	709.77	555.56	100	2 627.58	4 529.19± 79.79	6 073.13	2 985.25	100
ENGERIX-B	10.95	22.40±10.52	43.65	1.15	100	475.46	557.00±50.55	659.08	454.91	100	2 272.72	3 523.93±800.56	5 140.71	1 907.14	100
TWO-WAY ANOVA		NS (Between the groups)					NS (Between the groups)					NS (Between the Groups)			

GMT – geometric mean titer, AMT – arithmetic mean titer, SEM – standard error of mean , NS – non-significant .

100% seroconversion response on the 30th d after the 1st injection in both male and female groups. The sero-protection data also reported a 41.2-65.6% response which reached 100% on the 60th d after first injection. Descriptive statistical analysis also showed a geometric mean titer value of 13.77 mIU/mL in the BEVAC group and 10.95 mIU/mL in the ENGERIX-B group on the 30th d after the 1st injection (Table 2). The 60th d response showed a GMT value of 519.84 mIU/mL in the BEVAC group and 475.46 mIU/mL in the ENGERIX-B group (Table 2). On the 90th d, the antibody titre response observed was 2 627.58 mIU/mL in the BEVAC group and 2 272.72 mIU/mL in the ENGERIX-B group. No statistically significant difference was observed between the two experimental groups as well as between the sexes (Table 2).

Evaluation of reactogenicity

Two subjects in each group experienced pains at the injection site after the first vaccination. A total of six subjects in both groups showed a solicited adverse reaction, including pain, swelling and redness at the injection site. Three subjects in group B had a pain at the injection site after the third dose. No other serious adverse events occurred and no dose-related local or general symptoms were found during the study.

DISCUSSION

Immunization of susceptible persons against hepatitis-B is necessary to prevent not only acute diseases, but also the conversion and chronic states of hepatitis B virus infection. The initial immune response to the vaccines following the basic immunization series, is an important determinant of the duration of immunity. In the present study, all the subjects responded satisfactorily with an antibody titer >20 mIU/mL and also showed an 100% seroprotection response within 60 d after vaccination. Seven subjects also showed a rise of antibody titer within the level of 10 001-100 000 mIU/mL. Published studies regarding the dose-response relationship in terms of immunogenicity and sero-protection are highly varied. Chiaramonte *et al*[6] reported that the sero-protection reached a level of 99.6% within one month after primary immunization with the recombinant hepatitis-B vaccine. The findings of Assateerawatt *et al*[7] and Just *et al*[8] also were the same.

During the present study, an augmented vaccination schedule was adopted (0, 1, 2 mo). Clinical studies by Jilg *et al*[9], Hadler *et al*[10], Scheiermann *et al*[11] have clearly shown that there is no significant difference in level of immunogenicity between 0-1-2 mo and 0-1-6 mo schedules used for the immunization purpose. On the other hand, Marsano *et al*[12] have established that a 0-1-2 mo schedule could exert a quicker and identical rate of sero-protection in comparison to the standard schedule of 0, 1 and 6 mo. Wahl *et al*[13], Iu *et al*[14], and Hollinger[15] noticed significant and rapid protective antibody levels in accelerated immunization. In conclusion, BEVAC is safe, efficacious and immunogenic in comparison with the well documented ENGERIX-B.

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