Safety and Immunogenicity of a New Mammalian Cell-Derived Recombinant Hepatitis B Vaccine containing Pre-S_1 and Pre-S_2 Antigens in Adults*

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Abstract

Background: Previous data showed that new recombinant hepatitis B virus vaccine, which contains the S-protein component of the HBV surface together with the Pre-S_1 and Pre-S_2, is considerably more immunogenic than a second-generation recombinant HBV vaccine.

Objectives: To compare the immunogenicity and safety of a novel recombinant HBV vaccine S_1, Pre-S_1 and Pre-S_2 protein components of the hepatitis B surface antigen – Bio-Hep™, 10 μg dose, to a licensed vaccine containing only the S-protein component – Engerix-B, 20 μg dose.

Methods: A prospective randomized study included 524 adults – 260 in the Bio-Hep group and 264 in the Engerix-B group. Both vaccines were administered in a three-dose regimen given at 0, 1 and 6 months, and adverse events were recorded on a diary card 5 days after each vaccination. Immunogenicity was tested by measuring anti-hepatitis B surface antibody.

Results: One month after the third injection, 98% of the BioHep™ subjects were found to be seroprotected vs. 85.1% of the Engerix-B group. In addition, the geometric mean titers were 2,203 mIU/ml and 326 mIU/ml in the Bio-Hep-B and Engerix-B groups respectively. An immunogenic advantage of Bio-Hep-B was suggested by the rapid onset of antibody response – 66.5% were seroconverted one month after the first injection as compared to 19.3% in the Engerix-B group. No unexpected adverse events were observed, and the recorded events were mild in both groups.

Conclusions: BioHep™, a novel recombinant HBV vaccine containing S, Pre-S_1 and Pre-S_2 protein components, at a lower dose, is safe and more immunogenic than the conventional HBV vaccine that contains only S protein.

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HBV = hepatitis B virus

Hepatitis B virus infection is a major problem that effects both children and adults worldwide. Nearly 10% of adults infected by HBV do not recover and become HBV carriers. Long-term HBV infection can lead to chronic hepatitis, cirrhosis and primary hepatocellular carcinoma [1].

The two most commonly used recombinant vaccines in the western world [2,3] are rH-B-Vax (Merck & Co., USA) and Engerix-B (Smith Kline Biologicals, Belgium). These two vaccines contain only the non-glycosylated SHBs P24 [4] and share similar physical properties. However, their immunogenicity differs, and the recommended adult or pediatric dose per injection for Engerix-B® is double the recommended dose for rH-B-Vax®.

The biological function and the relative significance of the immune response to each of the envelope proteins (S, Pre-S_2 and Pre-S_1) has been shown [5] but is only partially understood. Recent studies have shown that the Pre-S antigens, particularly Pre-S_1, express highly immunogenic T and B cell epitopes, an important property for the immunogenicity and protection of third-generation vaccines [6,7]. Several studies have suggested that Pre-S_2 has the following properties: a) Pre-S_2 domain binds polymerized human serum albumin *in vitro*, b) synthetic peptides derived from a Pre-S_2 sequence may protect chimpanzees against HBV challenge [8], and c) Pre-S_2 has a domain that can also act as a B cell epitope. These combined properties seem to contribute to the enhanced immunogenicity of Pre-S_2 [9,10] in both forms.

It has been suggested that the Pre-S_2 antigen is an important factor in the penetration of HBV into its target cell, which may be prevented through an adequate anti-Pre-S_2 response. The Pre-S_1 domain of the envelope protein may play a dominant role in binding the virus to the hepatocyte.

Milich and co-workers [11] have recently shown that non-responsiveness to SHBs immmunization in mice can be circumvented through immunization with Pre-S proteins. Furthermore, immunization with synthetic MHSb (Pre-S_2 or Pre-S_2/S) peptides, as well as with yeast and Chinese hamster

ovary mammalian cell derived from Pre-S_2/S vaccines, may induce neutralizing antibodies and protect chimpanzees against HBV challenge [8,12].

There is justification for producing more immunogenic vaccines that could eventually be efficacious in groups of genetically or age-determined non-responders to yeast-derived vaccines, as well as in immune-suppressed and dialysis patients who do not respond adequately to conventional immunization. A more immunogenic vaccine may also enable a reduction in the number of injections required for long-term protection against HBV.

A third-generation mammalian cell-derived vaccine was first developed at the Pasteur Institute in transfected CHO cells, using cells that express S and Pre-S_2 antigens [13]. The new recombinant vaccine elicited higher seroconversion rates (94%) as compared to the plasma-derived vaccine (76%) in this population of vaccine-resistant patients [14].

Bio-Hep-B (Bio-Technology General [Israel] Ltd., Rehovot, Israel) is a novel recombinant vaccine produced via expression of the Pre-S_1 and Pre-S_2 and S protein components of HBsAg in CHO cells. The process yields particles that resemble circulating 22 mm particles in their protein composition and glycosylation pattern and harbor all antigenic epitopes and domains of the HBV envelope [15]. Several studies conducted in adults, children and neonates showed that this vaccine is highly immunogenic and safe [16]. The aims of the present study were to corroborate the safety and immunogenicity of Bio-Hep-B and to compare it to a licensed recombinant vaccine (Engerix-B, Smith Kline Biologicals, Rixensart, Belgium) in healthy adults.

Patients and Methods

Vaccine

Bio-Hep-B consists of HBsAg particles of the adw2 serotype, biosynthesized via recombinant DNA technology in engineered CHO cells harboring the entire *HBs* gene and manufactured according to Good Manufacturing Practice Guidelines of the Food and Drug Administration (USA) by BioTechnology General (Israel). The recombinant HBsAg are secreted into the culture medium as particles harboring the three surface proteins of HBV – S, Pre-S_1 and Pre-S_2 – in glycosylated and non-glycosylated form and in proportions that mimic the composition of authentic plasma-derived particles. Physico-chemical parameters of the recombinant HBsAg, including their size of 22 nm, a density of 1.17–1.20 g/ml and a 46% lipid content, also correspond to those of plasma-derived particles [2]. The manufacture, recovery and purification (98%) of the recombinant HBsAg is achieved by a non-disruptive proprietary process, which included media clarification, ultrafiltration and ion-exchange chromatography. Safety is assured by analysis for impurities such as endotoxin, microorganism (*Mycoplasma*, bacteria and fungi) and DNA (all within FDA guidelines) with inactivation of any adventitious virus by a formaldehyde step. Moreover, the Master Cell Bank is assured of safety with respect to tumorogenicity and presence of viruses. Vaccine formulation is achieved by alum absorption and addition of 0.01% thimerosal as preservative. Each batch of the formulated vaccine is also tested for immunogenic potency by seroconversion analysis in mice.

The control vaccine was Engerix^{TM}-B (manufactured by Smith Kline Biologicals, Rixensart, Belgium).

Study design

This prospective, randomized open-labeled study was performed in two medical centers in Israel (HaEmek in the north and Kaplan in the south), and included healthy, previously unvaccinated adults (aged 18 years). After informed consent was obtained the patients were randomized to receive (1:1) either Bio-Hep 10 μg/dose or Engerix-B 20 μg/dose. One milliliter of both vaccines was injected intramuscularly in the deltoid area at day 0, 30 ± 5 and 180 4 days. Blood samples were obtained at days 0, 30 ± 7,210 5 and 360 ± 30 days. Screening on day 0, included HBV markers (anti-HBc, HBsAg, anti-HBs) and alanine aminotransferase. ALT was also repeated at the last follow-up visit (360 ± 30 days). Only patients with negative HBV markers and normal ALT were included.

Immunogenicity was assessed by determination of anti-HBs titers by using a commercial radioimmunoassay kit (AV SAB, Abbot Laboratories, Chicago, IL, USA) and calculated by the Hollinger formula in international units per liter (mIU/ml) vs. a WHO reference standard. Seroconversion was defined as anti-HBs levels ⩾ 2.1 mIU/ml. Seroprotection was defined as anti-HBs levels ⩾ 10 mIU/ml.

Safety was assessed throughout the study by monitoring for adverse events and recording local signs and symptoms on a diary card for 5 successive days following vaccination.

Statistical analysis was performed using chi-square, Mann-Whitney-Wilcoxin test of proportions, Fisher's exact test and Student's *t*-test.

Table 1. Demographic characteristics

	Bio-Hep group	Engerix-B group
No. of patients	260	258
Dropped out	11	12
Age (yr)		
Mean (SD)	42.810.0	42.99.9
Min-Max	18–60	20–60
Gender		
Males	132	136
Females	128	128
Weight (kg)		
Males (mean SD)	77.712.5	78.913.4
Min-Max	49 – 125	56 – 155
Females (mean SD)	66.011.8	66.213.2
Min-Max	49-120	43-100

CHO = Chinese hamster ovary
HBsAg = hepatitis B surface antigen
anti-Hbs = anti-hepatitis B surface antibody
ALT = alanine aminotransferase

Results

Study population

Table 1 shows the characteristics of the study population by age, weight and gender for vaccination group. There are no differences between either group by age, gender and weight.

Of the 524 subjects recruited (260 received Bio-Hep-B and 264 Engerix-B), 247 and 252 completed the study, and 11 and 12 in the Bio-Hep-B and Engerix-B group respectively dropped out.

Immunogenicity

Bio-Hep-B elicited a higher rate of anti-HBs seroconversion/seroprotection and reached higher geometric mean titers than Engerix-B at all visits [Figures 1 and 2]. Bio-Hep-B achieved 66.5% vs. 19.3% seroconversion one month after the first dose ($P < 0.001$), 99.6% vs. 96% ($P < 0.001$) 1 month after the second dose, and 99.6% vs. 92.3% 6 months after the third dose ($P < 0.001$).

One month following the first vaccination, 34.7% vs. 5.4% of the subjects were seroprotected in the Bio-Hep-B and Engerix-B groups respectively ($P < 0.001$). Ninety-nine percent of the Bio-Hep vaccinees were seroprotected following the third injection and this persisted until the 12th month, compared to only 85% of the Engerix-B vaccinees ($P < 0.001$).

The additional advantage of Bio-Hep-B vaccine was exhibited in higher geometric mean titers – ranging from 4.9 mIU/ml at one month after the first dose, 9,254 mIU/ml one month after the third dose, to 2,203 mIU/ml one year later – as compared to the Engerix-B vaccine whose titers ranged from 0.2 mIU/ml to 1,812 mIU/ml and 326 mIU/ml, respectively.

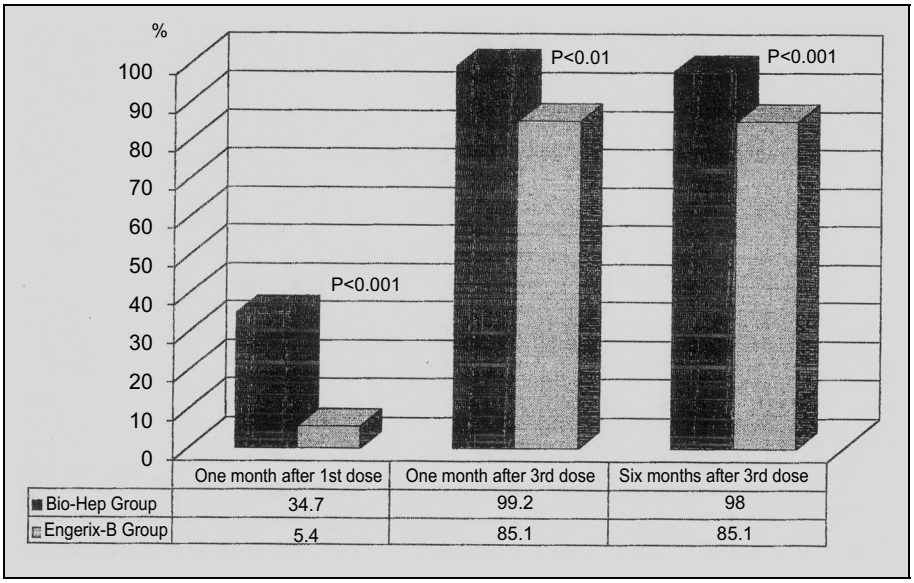

Figure 1. Seroprotection in both groups of vaccinated patients, defined as anti-HBs 10 mIU/ml.

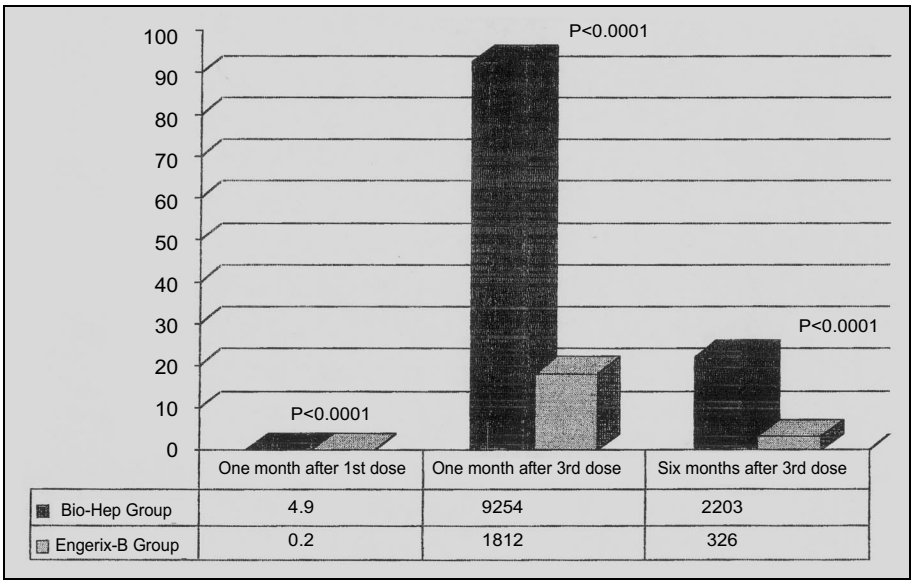

Figure 2. Geometric mean titers in both groups of vaccinated patients. GMT (95% CI) (mIU/ml)

Safety

Adverse events in both vaccination groups were similar [Tables 2 and 3]. Local signs and symptoms were generally mild and transient, lasting for 1–2 days after vaccination and consisting mainly of pain upon pressure, pain upon movement, and redness at the injection site. Moderate events were also similar in both groups and are presented in Table 3. No serious adverse events were reported in the Bio-Hep-B and Engerix-B vaccinated patients.

Table 2. Frequency of vaccine-related adverse events

Adverse experience	Bio-Hep-B	Engerix-B
Asthenia	Rigors	4
Dizziness		2
Fatigue		5
Fever		3
Headache		2
Hematoma within injection site		-
Malaise		-
Myalgia		2
Nausea		4
Pharyngitis		

Table 3. Incidence of local signs/symptoms

Local adverse effects	Post-dose 1		Post-dose 2		Post-dose 3		Total		P
	Bio-Hep-B n = 260(%)	Engerix-B n = 263(%)	Bio-Hep-B n = 255(%)	Engerix-B n = 261(%)	Bio-Hep-B n = 251(%)	Engerix-B n = 254(%)	Bio-Hep-B n = 766(%)	Engerix-B n = 778(%)	
Pain upon pressure	56 (21.5)	39 (14.8)	30 (11.7)	23 (8.8)	33 (13.5)	27 (10.6)	119(15.5)	89 (11.4)	0.1907
Pain with movement	32 (12.3)	18 (6.8)	32 (12.5)	14 (5.3)	15 (5.9)	13 (5.1)	79 (10.3)	45 (5.7)	0.0559
Pain strong enough to disrupt sleep	6 (2.3)	3 (1.1)	6 (2.3)	3 (1.1)	1 (0.4)	3 (1.1)	13 (1.7)	9 (1.1)	0.6060
Redness at injection site	40 (15.3)	23 (8.7)	12 (4.7)	10 (3.8)	12 (4.7)	8 (3.1)	64 (8.3)	41 (5.2)	0.1649
Itching at injection site	9 (3.4)	7 (2.6)	9 (3.5)	6 (2.3)	9 (3.6)	11 (4.3)	27 (3.5)	24 (3.0)	0.7812

Discussion

The present study corroborates previous data showing that a new recombinant hepatitis B virus vaccine that contains the S-protein component of the HBV surface together with the Pre-S_1 and Pre-S_2 is considerably more immunogenic than a second-generation recombinant HBV vaccine [17].

Several studies have been carried out in mice [18], neonates and children, as well as in adults [19,20]. While the exact reasons for the improved immunogenicity of Bio-Hep are not completely understood, it is probably the result of the spherical structure obtained by producing the vaccine in an animal-derived cell line (CHO) and from the presence of the Pre-S_1 and Pre-S_2 proteins on the surface of the vaccine particle [21].

The importance of Pre-S domains in the immunogenicity of HBV and the beneficial effect of an anti-Pre-S response in protective immunity have recently gained wide acceptance [22]. It should be noted that in view of the hydrophilicity of the Pre-S domains as compared to S, and their sensitivity to pepsin treatment, they are believed to be exposed at the particle surface and are thus highly accessible to the immune system [7]. Synthetic peptides corresponding to Pre-S_1 and Pre-S_2 elicit specific antibody production in mice and recognize distinct antibodies in infected human sera.

Both the Pre-S_1 and Pre-S_2 domains have been implicated in hepatocyte cell binding and viral penetration – Pre-S_2 by virtue of binding to polymerized human albumin, which binds to its receptor on liver cells [22], and Pre-S_1 by virtue of a specific hepatocyte cell-binding sequence localized to its amino terminus. The concept of an attachment blockage pathway of virus neutralization suggests that an effective immune response to a virus surface protein sequence involved in target cell recognition is an important component of the host's virus-neutralizing mechanism. Hence it is proposed that the Pre-S_1 and Pre-S_2 sequences involved in such receptor recognition are important in immunization.

In addition to a humoral response, protective immunity requires an effective cellular response consisting of memory and antigen-presenting and cytolytic T cells. In fact, both the Pre-S_1 and Pre-S_2 domains have been shown to harbor T cell-specific antigenic determinants that are highly relevant in vaccine preparations.

The importance of Pre-S_2 in protective immunization has been suggested following analysis of the response of various mouse strains to immunization with HBsAg consisting solely of S particles as compared to HBsAg containing 10–20% Pre-S_2 [22,23]. A mouse strain genetically unresponsive to immunization with particles consisting of S alone produced antibodies not only to the Pre-S_2 epitopes but also to S, upon immunization with Pre-S_2 containing HBsAg. Pre-S_1 inclusion in HBsAg has also been shown to circumvent non-responsiveness to vaccines harboring S alone or Pre-S_2 and S in specific mouse strains. In a similar way, Bio-Hep-B has recently been shown to provide a remarkable anti-HBs response in otherwise non-responsive mice [24].

We did not test the anti-Pre-S_1 and Pre-S_2 antibodies. The presence of these antibodies may be important in preventing infection by mutant strains in which the S protein is not affected by anti-HBs produced by vaccination. In chimpanzees, prevention against experimental HBV infection was achieved by using a synthetic peptide involving the product of the Pre-S_2 region [8,12]. In the future, testing and using vaccines containing more than one HBV component will necessitate routine use of additional tests for anti-HBs.

In conclusion, both vaccines were well tolerated with infreqeuntly reported adverse events, all of which were mild and subsided rapidly. Bio-Hep at a reduced dose is as safe as and more immunogenic than Engerix-B. The specific protective role of the Pre-S_1 and Pre-S_2 components needs to be studied further.

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