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Comparison of Immunogenicity in Balb/C Mice of Commercially Available Recombinant Hepatitis B Vaccines in Iran

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In this study the immunogenicity of four commercially available hepatitis B recombinant vaccines in Iran was compared. The vaccines included both the well known brand of Engerix-B and three biosimilars of Heberbiovac HB, Euvax B and Hepavax-Gene. Vaccines were administered intra-peritoneally (i.p) to Balb/C mice and the immune responses were evaluated by comparing the Geometric Mean Titer (GMT), the rate of seroconversion, seroprotection, ED50 as well as the relative potency of the vaccines. The GMT (mIU mL^{-1}) obtained for Heberbiovac-HB was at least six folds of the other vaccines while its ED50 (ng) was also the lowest among the tested formulations. Similar results were obtained when the seroconversion and seroprotection of Heberbiovac-HB was compared to the others. The relative potency of Heberbiovac-HB was 30.13 (μg $dose^{-1}$) which was well above the figures obtained for other vaccines. The results of immunogenicity markers in Balb/C mice of Heberbiovac-HB, a biosimilars hepatitis B vaccine in Iranian market was well above the well-known brand of Engerix-B but those figures obtained for other biogenerics were far behind those of Engerix-B. These results tend to suggest the important role of post marketing surveillance studies conducted by the national regulatory authorities in selecting the right products in national immunization programs.

Key words: Commercially available, hepatitis B vaccine, Balb/C mice, immunogenicity

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INTRODUCTION

Hepatitis B is a necro-inflammatory liver disease with variable severity. It causes morbidity and mortality world-wide due to its high incidence rate and lack of any definite cure (Leroux-Roels *et al*., 2000; Otağ, 2003). At present there are two billion infected people (Halperin *et al*., 2006) and WHO estimates that there are over 350 million chronic HBV carriers round the world (Leroux-Roels *et al*., 2000). Its mortality is as high as 1-2 million deaths in the world annually (Rebedea *et al*., 2006). Exposure of healthy adults to HBV results in a protective antibody response in 90-95% of cases, associated with either asymptomatic or acute clinical courses (Shokrgozar and Shokri, 2002). The risk of chronicity varies according to the age at the onset of infection, with rates of 90-95% in those infected perinatally (Gow and Mutimer, 2001) and 5-10% in adults throughout their lifespan. Host genetic factors are very important in the outcome followed by infection (Thursz *et al*., 1995). Chronicity rate is usually increased in those with immunosuppression. Persistent infection is associated with a healthy chronic carrier state in about 33% of cases and with chronic liver disease that can lead to liver cirrhosis and hepatocellular carcinoma in 67% of cases. The cellular immune response includes the elimination of virus which could lead to liver damage by the lytic activity of HBV-specific cytotoxic T lymphocytes (CTL) and production of inflammatory cytokines (Halperin *et al*., 2006; Guidotti and Chisari, 2006). Vaccination is the best available mechanism against hepatitis B infection (Otağ, 2003). Prophylactic vaccination leads to interrupting the transmission, reducing the pool of infected individuals and preventing the long-term sequelae of chronic liver disease. Vaccination with surface antigen of HBV (HBsAg) has been found to induce a protective antibody response in a similar proportion of the normal adult population and also in neonates and children (Shokrgozar and Shokri, 2002). These vaccines are extremely pure and are adjuvanted with aluminum compounds (Ascherio *et al*., 2001). The main objective of hepatitis B immunization strategies is to prevent chronic hepatitis B virus (HBV) infection and its serious consequences while therapeutic vaccination for chronic carriers has yet to be successful. As the patent for Hepatitis B vaccine has already expired, several biosimilar products have enrolled into the health care system of the countries around the world. The cheap price of the biogenerics is quite important for the national authorities of those countries who have the vaccine in their national immunization program (NIP). The aim of this study was to compare the immunogenicity of the commercially available recombinant hepatitis B vaccines in Iran. Immunogenicity of the vaccines were assessed by comparing the geometric mean titers (GMTs), rates of seroconversion and seroprotection, ED50 and relative potency in Balb/C mice after 28 days of intera peritoneum (i.p.) injection of the vaccine. The minimum protection (seroprotection) level considered was 10 mIU mL^{-1} while antibody responses between 1 and 10 mIU mL^{-1} were referred as seroconversion (Averhoff *et al*., 1998; West and Calandra, 1996).

MATERIALS AND METHODS

Vaccines: This study was conducted in Department of Drug and Food Control, Tehran University of Medical Sciences in 2007. The vaccines used in this study included Engerix-B (GSK, Belgium, Lot No. AHBVB127AG GS), Heberbiovac HB (Heberbiotech, Cuba Lot No. 4C322/0), Euvax B (LG Chemicals, Korea, Lot No. WVA05019), Hepavax-Gene (Green-Cross Vaccine Corporation, Korea, Lot No. 2063222). All the vaccines contained 20 μg mL^{-1} of HBs-Ag adsorbed onto aluminum hydroxide. The vaccines were administered intra-peritoneally (i.p) to Balb/C mice. Dilutions of 1:512 (0.03906 μg mL^{-1}), 1:64 (0.3125 μg mL^{-1}) and 1:8 (2.5 μg mL^{-1}) of individual vaccines were prepared in phosphate buffer, pH 7.4 containing of the relevant aluminum adjuvant with the equal concentration of aluminum in vaccine preparations. Engerix-B hepatitis B vaccine was used as reference to estimate the relative potencies of the vaccines. Phosphate buffer containing equal concentration of aluminum hydroxide with no vaccine was served as negative control.

Animals: Mice of the female Balb/C C3H strain were obtained from Charles River Laboratories (Germany) and housed in Micro-Isolator™ in 25°C, 12 h day and night cycle with 50±5% of relative humidity. Food (5 g) and water (6 mL) were served, for each mouse daily. Mice were generally 5-6 weeks old at the start of experiments. All studies were performed in accordance with the procedures issued by the Institutional Animal Care and Use Committee. Each dilution of vaccine was injected to 15 mice. The injection volume was 1 mL for each mouse and the rout of injection was i.p. After 28 days following the injection the blood samples were collected from the heart of anaesthetized animal for detecting HBs antibody titer. The serum of the blood samples were separated by centrifuging at 3000 x g for 10 min. The serum was stored at -20°C before determining of antibody titer.

Determination of anti HBs titers: Anti HBs antibody was determined by ELISA technique using Diasorin, ETT-AB-AUK-3 anti-HBs antibody ELISA kit (Italy). The seroprotection level was achieved when the antibody titer was at least 10 mIU mL^{-1} and antibody response between 1 to 10 mIU mL^{-1} was considered as seroconversion (Averhoff *et al*., 1998).

Statistics: ED50 for each formulation was evaluated by SPSS VER.13 using Probit method while the relative potencies of formulations were evaluated using quantal responses method (British Pharmacopoeia, 2007). Geometric mean anti-HBs Ag titres (GMTs) were calculated by taking the anti-log of the mean of the log titre transformations. Antibody titres below the cut-off the assay were given an arbitrary value of half the cut-off for the purpose of GMT calculation.

RESULTS

Seroconversion of the vaccines: The results of 28 days seroconversion rates of various dilutions of different vaccines containing aluminum hydroxide and also those of the negative control are shown in Fig. 1. All of the vaccines showed 100% of seroconversion at concentrations of 2.5 μg mL^{-1} and more while at lower HBsAg concentrations (0.31, 0. μg mL^{-1}), Heberbiovac-HB showed the highest seroconversion rate (100, 40%) compared to Engerix-B (86.66,33.33%), Euvax-B (73.33, 20%) and Hepavax_Gene (40, 6.66%).

ED50: ED50 which is the dose induces seroconversion in 50% of vaccinated population was calculated for individual vaccines with statistical software package SPSS VER.13 using probit method and the results were 81.09, 137.53, 196.17 and 368.02 ng for Hebrbiovac-HB, Engerix-B, Euvax-B and Hepavax-Gene respectively. The lowest ED50 obtained for Heberbiovac-HB which corresponds to better immunoginicity.

Seroprotection of the vaccines: Seroprotection rates of various dilutions of HBs vaccine formulations containing aluminum hydroxide and also those of the negative control are shown in Fig. 2. All the formulations except Hepavax-Gene showed 100% of seroprotection at concentrations of 20 μg mL^{-1} which showed 93.33% of seroprotection. At lower HBsAg concentrations (2.5, 0.31, 0.039 μg mL^{-1}), Heberbiovac-HB showed the highest seroprotection rate (100, 100, 20%) compared to Engerix-B (100, 66.66, 0%), Euvax-B (66.66, 53.33, 0%) as well as Hepavax_Gene (46.66, 20, 0%).

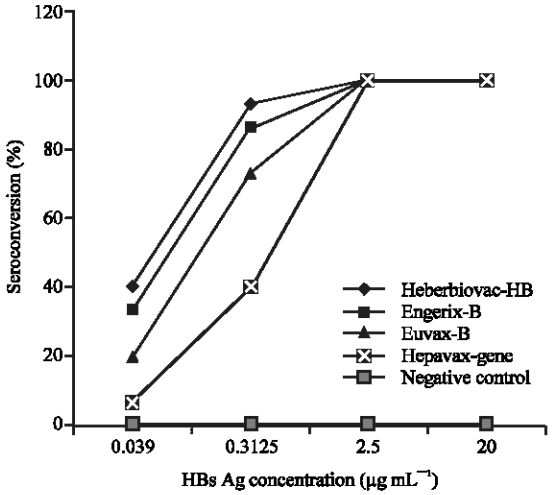

Fig. 1: Seroconversion rates of different aluminum hydroxide adjuvanted hepatitis B vaccine preparations available in Iran after 28 days of i.p. injection in Balb/C mice. Phosphate buffer containing aluminum hydroxide with on vaccine was considered as negative control

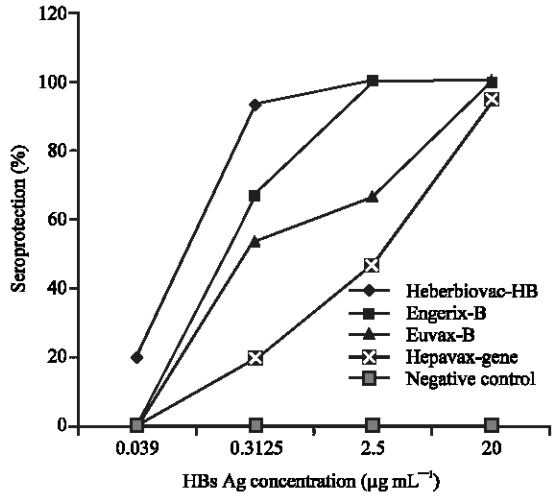

Fig. 2: Seroprotection rates of different aluminum hydroxide adjuvanted hepatitis B vaccine preparations available in Iran after 28 days of i.p. injection in Balb/C mice. Phosphate buffer containing aluminum hydroxide with on vaccine was considered as negative control

Geometric mean antibody titers (GMTs): The geometric mean antibody titers of different hepatitis B vaccine preparations are shown in Table 1. Using 20 μg mL^{-1} antigen concentration, the GMT obtained with Heberbiovac-HB was 8646.61 mIU mL^{-1} which is

Table 1: GMT rates of different aluminum hydroxide adjuvanted hepatitis B vaccine preparations available in Iran after 28 days of i.p. injection in Balb/C mice

Vaccine	Dose (μg mL^{-1})	GMT (mIU mL^{-1})
Heberbiovac-HB	20.00	8646.61
	2.50	658.41
	0.3125	63.56
	0.03906	3.02
Engerix-B	20.00	1341.68
	2.50	437.18
	0.3125	8.94
	0.03906	2.12
Euvax-B	20.00	1188.86
	2.50	47.08
	0.3125	7.92
Hepavax-Gene	20.00	366.06
	2.50	12.46
	0.3125	3.47
	0.03906	1.59

Table 2: Relative potency and its lower limit and upper limit of different hepatitis B vaccine preparations after 28 days of i.p. injection in Balb/C mice

Formulation	Lower limit	Relative potency	Upper limit
Heberbiovac-HB	23.02	30.13	39.73
Euvax-B	7.09	11.13	17.07
Hepavax-Gene	3.08	4.63	6.71
Engerix-B	-	20.00	-

The upper confidence limit (p = 0.95) of the estimated relative potency is not less than 1.0

significantly (six folds) above the figures obtained for other products with Engerix-B (1341.68 mIU mL^{-1}), Euvax-B (1186.86 mIU mL^{-1}) and Hepavax_Gene (366.06 mIU mL^{-1}). The highest GMT titer was observed at lower concentrations of antigen for Heberbiovac-HB too.

Relative potency: Relative potency of formulations was determined using quantal responses method based on the seroconversion figures obtained for individual vaccine preparations (British Pharmacopoeia, 2007). Engerix-B was used as the reference vaccine. Relative potency, lower limit and upper limit of relative potency of the vaccines are shown in Table 2. The relative potency obtained for Heberbiovac-HB (30.13 μg/dose) was significantly higher than other vaccines.

DISCUSSION

Post marketing surveillance studies on the biological products in the developing countries are rare. To our knowledge, this is the first reported comparative study between the four commercial hepatitis B vaccines used in Iran. Safe and effective vaccines against hepatitis B virus infection have been available for over a decade (Safary and Andre, 1999) and currently HBV vaccination has been included in the routine child hood vaccination program in more than 100 WHO member states (Kane, 1998). Previous reports conducted in the United States showed significant differences in the magnitude of anti body responses (GMTs) using the Engerix-B and Recombivax vaccines in different human vaccinated groups and the seroprotection levels (above 10 mIU mL^{-1}) were 83-100% and 69-99%, respectively (Wood *et al*., 1993). Similar results have been reported with recombinant vaccines throughout the world targeting different risk groups (Wood *et al*., 1993). Therefore about 17 or 31% of the population, depending on the vaccinated groups, may remain non-protected. On the other hand for high-risk groups such as healthcare workers it is suggested that one should aim for levels above 100 mIU mL^{-1} (Wood *et al*., 1993; Yuen *et al*., 1999). Also long-term follow-up studies have shown that the duration of vaccine-related immunity declines after several years. A number of such studies, where monitoring continued up to 12 years after vaccination, showed that anti-HBs levels declined over time and that half of the vaccinated persons had levels below 10 mIU mL^{-1} (Williams *et al*., 2003; Zinkernagel *et al*., 1997). Protective immunity correlates with either the duration of antigen persistence or with the extent of clonal expansion of T cells during the early immune response which determined by the initial antigen dose (Zinkernagel *et al*., 1997). Therefore the type of vaccine has great effect on the amount of related immune response and its durability.

In this study the immunogenicity of four commercially available aluminum hydroxide formulated recombinant hepatitis B vaccines containing 20 μg mL^{-1} of HBs Ag of vaccine were assessed in Balb/C mice. The GMT figures obtained for Heberbiovac-HB was about six folds higher than Engerix-B and its ED50 was the lowest between the formulations. Similar results were obtained for seroconversion and seroprotection. The relative potency figure of Heberbiovac-HB was also an indication of better immunogenicity among the other tested products.

These results tend to suggest the important role of *in vivo* post marketing surveillance studies, which may show different results from in-vitro studies, conducted by the national regulatory authorities in selecting the right products in national immunization programs.

Vaccine preparations with higher immunogenicity could induce more durable effect in the vaccinated groups in comparison to other vaccines. This topic is more important in high risk groups such as healthcare workers. Clinical studies pointing different age groups need to confirm the current data.

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