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Low dose revaccination induces robust protective anti-HBs antibody response in the majority of healthy non-responder neonates

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Summary A sizeable proportion (1–10%) of healthy adults and to lesser extent neonates vaccinated with triple 10 μg hepatitis B (HB) vaccine fail to mount a protective antibody response. Revaccination with the same vaccine dose has proved to be effective in a significant number of primary non-responders. The influence of revaccination with lower vaccine doses however has not been studied adequately in non-responder neonates. This study was conducted to evaluate the influence of supplementary vaccination with a single low and standard dose of a recombinant hepatitis B (HB) vaccine in healthy Iranian non-responder neonates to primary vaccination.

Iranian neonates unable to respond to primary vaccination with 10, 5 or 2.5 μg doses of recombinant HB vaccine were revaccinated with a single additional dose of the same concentration. Serum anti-HBs antibody titer was measured by sandwich ELISA. Administration of a single additional dose induced seroprotection (anti-HBs $\geq$ 10 IU/L) in 10/12 (83%), 10/12 (83%) and 21/24 (87.5%) of non-responder neonates in 10, 5 and 2.5 μg vaccine recipients with geometric mean titers (and 95% confidence limits) of 1358 (258–7142), 401 (79–2038) and 164 (62–433) IU/L, respectively. The log-transformed antibody titer obtained for the 10 μg dose recipients was significantly higher than that of the 2.5 μg dose vaccinees ($p = 0.028$). No significant differences in anti-HBs titer were observed between other groups of vaccinees. However, the total seroprotection rates obtained after administration of four low doses of 2.5 and 5 μg were significantly higher than that obtained after administration of the classical three 10 μg doses ($p = 0.029$ and $p = 0.006$, respectively). The total seroprotection rates were similar between all groups of vaccines receiving four doses of 2.5, 5 and 10 μg vaccine doses.

These results indicate that a significant proportion of non-responder neonates can be induced to develop a protective anti-HBs response following revaccination with a single low dose

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vaccine. Thus adaptation of four low dose (2.5 or 5 μg) vaccination is expected to induce higher seroprotection rate and lower or comparable anti-HBs antibody titer in healthy neonates.

Introduction

Hepatitis B virus (HBV) infection is a global health problem with an estimated chronic carrier rate of 350 million worldwide. Universal vaccination of neonates and children has been considered as the most effective strategy for control of HBV infection by World Health Organization (WHO) [1]. Recent epidemiological studies have clearly proved effectiveness of this strategy for control of asymptomatic carrier rate as well as prevalence of fulminant acute infection, cirrhosis and hepatocellular carcinoma [1—4]. More than a decade after the WHO recommendation to implement universal hepatitis B vaccination, 151 countries have included HB immunization into their national immunization program as a routine vaccine given to all infants and many additional countries are planning for mass vaccination in the next few years [1,5]. Vaccine expense is the most important factor that limits the use of HB vaccine for mass vaccination of neonates, particularly in developing countries [6,7].

Vaccination with the major surface antigen of HB virus (HBsAg) induces protective antibody response (anti-HBs $\geq$ 10 IU/L) in the majority of vaccinees, however, 1—10% of healthy neonates and adults fail to generate a protective antibody response to HB vaccine [1,8,9]. Vaccination failure is more frequent in healthy adults compared to neonates [8]. These non-responders remain susceptible to infection with HBV [10]. Several strategies have been undertaken to overcome the unresponsiveness state, among these use of supplementary vaccine dose has received increasing attention [10]. We have previously demonstrated that administration of a single booster classical dose (10 μg) of a recombinant HB vaccine induces a protective antibody response in 90% of non-responder neonates receiving the primary triple vaccine doses [11]. We also observed a similar seroprotection rate following primary vaccination with low doses (2.5 and 5 μg) of recombinant HB vaccine compared to the classical 10 μg dose [12]. The effect of booster vaccination with low vaccine doses, however, has not been widely studied. This issue might have important implications in modification and optimization of the present vaccination scheme. This study was conducted to evaluate the influence of revaccination with a single low dose of 2.5 or 5 μg compared to the classical 10 μg dose of a recombinant HB vaccine in healthy Iranian non-responder neonates to primary vaccination of the same dose.

Materials and methods

Subjects and vaccination scheme

A total of 1380 healthy Iranian neonates were included in this study. Gestational age, birth weight and sex of the neonates were recorded and only physically healthy neonates with a minimum weight of 2500 g were enrolled into this study. A recombinant hepatitis B vaccine (Heberbiovac, Heberbiotec Co., Cuba) was administered into the quadriceps muscle at 0, 1.5 and 9 months intervals. Neonates were randomized into three groups according to the vaccine dosage: group 1 (n = 521, 219 males and 189 females), group 2 (n = 451, 258 males and 263 females) and group 3 (n = 408, 219 males and 189 females) received 10, 5 and 2.5 μg HB vaccine, respectively. The standard vaccine dose for neonates is 10 μg, according to the national vaccination program of Iran. Serum samples were collected 2—4 weeks after completion of the vaccination course and anti-HBs antibody was quantitated. The fourth vaccine dose of the same concentration was administered to non-responder neonates (anti-HBs < 10 IU/L) 1—3 weeks after completion of primary vaccination in each group (group 1: n = 12, 7 males and 5 females, group 2: n = 12, 7 males and 5 females, group 3: n = 24, 13 males and 11 females). Anti-HBs level was then measured in their serum 2—4 weeks after revaccination.

Detection of HBV markers

HBs antigen, anti-HBs and anti-HBc antibodies were detected by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Behring, Germany). Anti-HBs antibody was quantitated by a sandwich ELISA using appropriate dilution of a positive sample with a known concentration of anti-HBs expressed as IU/L, provided by the manufacturer.

Statistical analysis

Parametric analyses (t-test) were performed on log-transformed titers of anti-HBs antibody within and between different groups of vaccine recipients after primary and booster vaccinations. Comparisons were made between 2.5, 5 and 10 μg dose vaccinated groups (male with male, female with female and total with total) separately, after primary and booster vaccinations (between groups comparison) and also within each group of vaccinated neonates, between males and females (within group comparison). Seroprotection rates were analyzed within and between different groups using Chi-Square, Fisher's exact or McNemar test, as appropriate. p values of less than 0.05 were considered significant.

Results

After completion of primary vaccination 97.3%, 96.9% and 93.4% of vaccinees who received triple doses of 10, 5 and 2.5 μg of recombinant HB vaccine developed protective titer of anti-HBs antibody ($\geq$10 IU/L), respectively (Table 1 and Figure 1A). Seroprotection rates were similar in the 10 and 5 μg groups and were significantly higher in both groups than that observed in the 2.5 μg group (p < 0.05).

The GMTs (and 95% confidence intervals) of anti-HBs antibody were 3132 (2605—3766), 2045 (1677—2493) and 1115