This study aimed to find out the immune response effects and side effects after recombinant yeast hepatitis B vaccine inoculation in neonates. All 203 neonates were divided into three groups: 25 subjects in group A whose mother was HBsAg(+) HBeAg(+), 43 subjects in group B whose mother was HBsAg(+) HBeAg(-) and 135 subjects in group C whose mother was HBsAg(-). The immune response effects at 12th month after first inoculation were compared and side effects were observed. Non-responders received re-immunization at 12th month after first inoculation and immune response effects were compared at 36th month after first inoculation. There was no significant difference of anti-HBsAg(+) neonates proportion between group “A plus B” and group C, neither between group B and C at 12th month after innoculation. But there were significant differences between group A and B or C. There were 3, 1 and 0 HBsAg(+) neonates in 3 groups at 12th month after the first inoculation. There were 5, 2 and 10 infants identified as non-responders and received re-immunization in 3 groups. Among of the “non-responders” significant differences were found in anti-HBsAg(+) proportion between group “A plus B” and group C at 36th month. Only 1 HBsAg(+) was found in group A at 36th month. All subjects of responders remained anti-HBsAg(+) at 36th month. No serious side effects were found. This China-made recombinant yeast hepatitis B vaccination is safe and effective to prevent HBV vertical transmission. HBsAg and HBeAg status of mother perhaps affect the immune response effects on neonates.

**Key words:** Recombinant yeast hepatitis B vaccine, neonates.

**INTRODUCTION**

In areas of high hepatitis B endemicity, most of the population becomes infected by the virus usually in the perinatal period and childhood and 8~20% become chronic carriers (World Health Organization, 2000). In China, more than 1.3 hundred million are chronic HBV infections and nearly 60% were infected in infants. WHO suggests that the best way to bring down the prevalence of HBV infection is to introduce HBV vaccine into routine immunize inoculation (Kiran, 2004). In Taiwan, this has resulted in a drop in carriage from 10.5% prior to vaccination, to 6.3% with the selective program, to 1.7% with universal newborn vaccination (Hsu et al., 1999). It also resulted in a significant reduction in the incidence of hepatocellular carcinoma (Ma et al., 2007).

Hepatitis B virus (HBV) infection is common in China, especially in Guangdong province (Wu et al., 2009). Vertical transmission of HBV is an important route of its prevalence, and HBV vaccination is the main means to prevent its spreading (Lixia et al., 2007). This study aimed to find out the safety and immunity effects after recombinant yeast hepatitis B vaccination in newborns. First generation vaccines were plasma-based using a purified hepatitis B surface antigen. Research of the plasma-derived hepatitis B vaccine began in the late 1960s and it was licensed in 1981. In 1983 a universal hepatitis B vaccination program for newborns was initiated in China towards eradication of the infection, only involving the newborns of HBsAg-positive mothers. Now the program was extended to all newborns. During the decade since the vaccination program was implemented, the
prevalence of HBsAg among persons younger than 15 years of age decreased from 9.8 to 8.2% in China (Liang et al., 2009).

Plasma-derived hepatitis B vaccine prepared from 25nm HBV surface antigen particles from chronic HBV carriers' plasma (Szmuness et al., 1981). So safety of plasma-derived hepatitis B vaccine remains a hidden danger. Furthermore, the high cost of plasma-derived hepatitis B vaccine could not meet the needs of universal HB vaccination program in China. Later yeast recombinant HB vaccines were introduced for clinical use in 1986. Now the yeast recombinant HB vaccine made in China had been put into using in the whole country.

MATERIALS AND METHODS

Subjects and grouping

The immune response effects and side effects of recombinant yeast HBV vaccine were observed and studied prospectively. All 203 neonates were born in our hospital from 2004-2009, and were divided into three groups:

1. 25 subjects in group A whose mother was HBsAg(+) HBeAg(+).
2. 43 subjects in group B whose mother was HBsAg(+) HBeAg(-).
3. 135 subjects in group C whose mother was HBsAg(-).

All of HBsAg(+) mother did not receive anti-HBV therapy. All families of the neonates included gave written informed consent to participate in this study and conformity certification was confirmed by Ethics committee.

Source of vaccines

The yeast recombinant hepatitis B vaccine (10 and 20µg) was produced by Shenzhen Kangtai Biological Products Co Ltd in China (Batch No: 20051131, 5µg/0.5 mL). These vaccines were used in validity duration.

Vaccine inoculation and re-immunization

Yeast-derived hepatitis B vaccine (10µg) was inoculated in the deltoid muscle for all neonates at 24 h, 1 and 6 months after birth. Re-immunization was performed on neonates who were considered as non-responders (negative for HBsAg and Anti-HBsAg by ELISA test) at 12th months after primary vaccination series. Non-responders were given the option of receiving an additional three-dose booster vaccination series which would be performed at 0, 1 and 2 months after 12th month using 20µg vaccine per dose. All vaccinations were also injected intramuscularly into the deltoid region of subjects.

Follow up and laboratory assays after vaccination

HBV markers including HBsAg, antibody to HBsAg (Anti-HBsAg) were detected by ELISA (the same method with detection of their mothers) at 12th and 36th month after birth for all the neonates.

Statistics analysis

The response rates of vaccination were compared and analyzed by SPSS 13.0 software using Chi-Square test. P < 0.05 was considered statistically significant.

RESULTS

Observation at 12th month

The total response rate was 89.66% (182/203) in all neonates at 12th months after the primary vaccination and among them there were 17, 40 and 125 responders in group A, B and C. There were significant differences of the response rate between group A and C, and even between group A and B. There was no significant difference of the response rate between group “A plus B” and C, neither between group B and C (Table 1). There were 8, 3 and 10 subjects been considered non-responders from 3 groups at 12th month respectively, and among them 3 and 1 neonates were found HBsAg positive in group A and B (no HBsAg positive neonates were found in those 10 subjects in group C ). There was significant difference in the rate of HBsAg positive in neonates between group “A plus B” and C, but no same difference was found between group A and B (Table 1).

Response rates

Comparison among all 3 groups: $\chi^2 = 14.422, P = 0.001$; Group “A plus B” compared with group C: $\chi^2 = 3.749, P = 0.007$; Group B compared with group C: $\chi^2 = 0.009, P = 0.0925$. Rates of HBsAg(+) in neonates after primary vaccination: Group “A plus B” compared with group C: (P = 0.012); Group A compared with group B: P = 0.137.

Table 1. Effects of vaccination at 12th month after birth in 3 groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Anti-HBsAg(+) (%)</th>
<th>Anti-HBsAg(-) HBeAg(+) (%)</th>
<th>HBsAg(-) (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17(68%)</td>
<td>3(12)</td>
<td>5(20)</td>
<td>25</td>
</tr>
<tr>
<td>B</td>
<td>40(93.01%)</td>
<td>1(2.33)</td>
<td>2(4.66)</td>
<td>43</td>
</tr>
<tr>
<td>C</td>
<td>125(92.59%)</td>
<td>0(0)</td>
<td>10(7.41)</td>
<td>135</td>
</tr>
<tr>
<td>Total</td>
<td>182(89.66%)</td>
<td>4(1.97)</td>
<td>17(8.37)</td>
<td>203</td>
</tr>
</tbody>
</table>

Note: Response rates: Comparison among all 3 groups: $\chi^2 = 14.422, P = 0.001$; Group “A plus B” compared with group C: $\chi^2 = 3.749, P = 0.007$; Group A compared with group C: $\chi^2 = 12.777, P = 0.005$; Group A compared with group B: $\chi^2 = 9.300, P = 0.007$; Group B compared with group C: $\chi^2 = 0.009, P = 0.0925$. Rates of HBsAg(+) in neonates after primary vaccination: Group “A plus B” compared with group C: (P = 0.012); Group A compared with group B: P = 0.137.
Table 2. Effects of re-immunization at 36th month in 3 groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Anti-HBsAg(+)</th>
<th>Anti-HBsAg(-)HBsAg(+)</th>
<th>HBsAg(-)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>1</td>
<td>13</td>
<td>17</td>
</tr>
</tbody>
</table>

Note: Response rates of re-immunization: Group “A plus B” compared with group C: $P = 1.000$.

Table 3. Effects of vaccination at 36th month after birth in 3 groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Anti-HBsAg(+) (%)</th>
<th>Anti-HBsAg(-)HBsAg(+) (%)</th>
<th>HBsAg(-) (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>17(68)</td>
<td>4(16)</td>
<td>4(16)</td>
<td>25</td>
</tr>
<tr>
<td>B</td>
<td>41(95.34)</td>
<td>1(2.33)</td>
<td>1(2.33)</td>
<td>43</td>
</tr>
<tr>
<td>C</td>
<td>127(94.07)</td>
<td>0(0)</td>
<td>8(5.93)</td>
<td>135</td>
</tr>
<tr>
<td>Total</td>
<td>185(91.13)</td>
<td>5(2.46)</td>
<td>13(6.41)</td>
<td>203</td>
</tr>
</tbody>
</table>

Note: Response rates: Comparison among all 3 groups: $\chi^2 = 18.947, P < 0.001$; Group “A plus B” compared with group C: $\chi^2 = 4.314, P = 0.038$; Group A compared with group C: $\chi^2 = 15.934, P < 0.001$; Group A compared with group B: $\chi^2 = 9.427, P = 0.002$; Group B compared with group C: $\chi^2 = 0.1, P = 0.752$. Rates of HBsAg(+) in neonates after re-immunization: Group “A plus B” compared with group C: $P = 0.004$; Group A compared with group B: $P = 0.053$; Group A compared with group C: $\chi^2 = 12.777, P = 0.005$; Group A compared with group B: $\chi^2 = 9.300, P = 0.007$; Group B compared with group C: $\chi^2 = 0.009, P = 0.0925$.

Rates of HBsAg(+) in neonates after primary vaccination

Group “A plus B” compared with group C: $(P = 0.012)$; Group A compared with group B: $P = 0.137$

Observation at 36th month

There were 5, 2 and 10 neonates that received the re-immunization and only 1 in group B and 2 in group C were found anti-HBsAg(+) at 36th month after birth. There was still no significant difference of the response rate between group “A plus B” and C (Table 2). And those who were responders with anti-HBsAg(+) didn’t turn to negative at 36th month. Moreover, the total response rate was 91.13% (185/203) in all neonates at 36th month after birth (after the primary vaccinations and re-immunization) and among them there were 17, 41 and 127 responders in group A, B and C finally.

There were significant differences of the response rate between group “A plus B” and C, even between group A and C or B. But, just like the result at 12th month, there was no significant difference of the response rate between group B and C. There was significant difference in the rate of HBsAg(+) in neonates between group “A plus B” and C, but no same difference was found between group A and B (Table 3).

Response rates

Comparison among all 3 groups: $\chi^2 = 18.947, P < 0.001$; Group “A plus B” compared with group C: $\chi^2 = 4.314, P = 0.038$; Group A compared with group C: $\chi^2 = 15.934, P < 0.001$; Group A compared with group B: $\chi^2 = 9.427, P = 0.002$; Group B compared with group C: $\chi^2 = 0.1, P = 0.752$.

Rates of HBsAg (+) in neonates after re-immunization

Group “A plus B” compared with group C: $P = 0.004$; Group A compared with group B: $P = 0.057$.

Safety of the vaccine

Mild local reactions such as pain in injection region occurred. But no major HBV vaccine-related adverse reactions were found in all the subjects.

DISCUSSION

HB vaccine has been successfully integrated into routine infant immunization in China, especially for infants in 24 h after birth, and the prevalence of HBsAg(+) has been greatly reduced among children born after 1990s (Liang et al., 2009). In this study, the total response rate was
89.66 and 91.13% at 12th and 36th month after birth, and no severe side effects were found. Even for the infants born from mothers of HBsAg positive, the response rate could reach at 83.82-85.29%. This has shown that the China-made HBV vaccine to be safe, immunogenic and efficacious in preventing transmission of hepatitis B virus. Because of the antibody remained at 36 months in our study, booster revaccination within 3 years seems unnecessary (Wu et al., 1999). At 12th month after birth and primary vaccination, the response rates were correlated with HBeAg status of the mother and the rates of HBsAg positive were correlated with HBsAg status of the mother.

Response rates of neonates in group A were the lowest and there was no significant difference between neonates from HBsAg(+) mother and neonates from HBsAg(-) mother. And the rate of HBsAg(+) neonates from HBsAg(+) mother was much higher than from HBsAg(-) mothers. But, it is so interesting that the similar phenomenon did not occur at 36th month after re-immunization. Response rates of neonates in group A were still the lowest and there was significant difference emerging between neonates from HBsAg(+) mothers and neonates from HBsAg(-) mothers. And rate of HBsAg(+) neonates from HBsAg(+) mothers was still much higher than from HBsAg(-) mothers. From this result, importance of re-immunization should be highlighted. Nevertheless, due to the small sample size, these findings should be verified by larger studies.

The chance of a child becoming chronically infected with HBV can be around 70 to 90% when a mother is positive for both HBsAg and HBeAg. And if the mother is positive for HBsAg but negative for HBeAg, the risk of transmission will be lower (Akhter et al., 1992). But our research did not get similar conclusions. In my results there were no significant differences between group A and B in the rates of HBsAg(+) in neonates after primary inoculation and re-immunization. Universal vaccination, which is safe and effective, is the only practical means of achieving eradication of hepatitis B (Olshen et al., 2007). Though hepatitis B vaccines has been proved to be effective in preventing HBV infections, some neonates still failed to produce protective antibodies from both HBsAg(+) and HBsAg(-) mothers in our study, even be infected by HBV.

Main causes of poor response to HBV vaccination, including intrauterine infection, vaccine escape mutants, genetic hypo- or no- responsiveness to HBsAg and HBV gene heterogeneity of the mother, have not been fully elucidated (Liu et al., 2009). Moreover, poor compliance of the vaccines may be one of the reasons. Off schedule vaccination, with less number of doses or long time off the recommended time schedule, may cause poor immune response to HBV vaccination (Chang, 2004). A few non-responders had seroconversion (anti-HBs positive) after re-immunization. So detecting anti-HBs titers should be routine examination after the primary vaccinations series to find out non-responders and reimmunization should be performed on them in time to improve the response rate. But a repeated dose of S-recombinants was unable to elicit a secondary anti-S antibody response in a few non-responders.

These findings do not support the assumption that the poor immunogenicity of some extrinsic antigens could be overcome by administering repeated doses of the recombinant (Kutinova et al., 1999). And Piyanit et al (2009) suggested it is important that immunization programs ensure timely second dose vaccination to infants born to mothers with chronic HBV infection. So, further efforts to improve compliance of the vaccinations, better vaccine with higher immunogenicity and more reasonable re-immunization strategy are needed.

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