Immunogenicity and safety of hepatitis E vaccine in healthy hepatitis B surface antigen positive adults

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A recombinant hepatitis E vaccine, Hecolin®, has been proven safe and effective in healthy adults. As hepatitis B surface antigen (HBsAg) positive individuals have a higher risk of poor prognosis after super-infection with hepatitis E virus (HEV), the safety and immunogenicity of Hecolin® in this population should be assessed. The present study is an extending analysis of data from a large randomized controlled clinical trial of Hecolin®. Healthy participants (n = 14 065) without current or previous evidence of chronic liver disease were randomized to receive Hecolin® or placebo (hepatitis B vaccine) and donated their blood samples before vaccination and subsequently over 31 mo. Most of the adverse events were mild and comparable between participants with and without baseline hepatitis B surface antigen (HBsAg). No vaccine-related serious adverse events were reported. Rates of serious adverse events in HBsAg (+) or HBsAg (-) participants were also comparable between both groups. Almost all participants in the Hecolin® group seroconverted to anti-HEV one month after full vaccination. The antibody response rates and levels were similar in HBsAg (+) and HBsAg (-) participants (98.38%, 19.32 Wu/mL vs. 98.69%, 19.00 Wu/mL). The two-year antibody dynamics of HBsAg (+) participants overlapped perfectly with those of HBsAg (-) participants. In conclusion, the safety and immunogenicity of Hecolin® for HBsAg (+) adults is very similar to that for the general population.

Introduction

Hepatitis E virus (HEV) is one of the leading causes of acute hepatitis.[1] An estimated one third of the world's population living in developing countries has been infected with HEV,[2] and reports of autochthonous hepatitis E cases are increasing in developed countries.[3-7] Recently, a striking spectrum of serious complications has been reported, including "acute-on-chronic" liver failure, extra-hepatic manifestations, and chronic hepatitis. These complications are mainly associated with autochthonous hepatitis E in developed countries.[8,9] Acute-on-chronic disease, which refers to hepatitis with the rapid appearance of signs of liver failure, ascites and encephalopathy in a person with pre-existing liver disease, is usually more severe than acute hepatitis

and sometimes progresses to fulminant hepatic failure, with a fatality rate of up to 70%.[10,11] Therefore, it is imperative for people with a higher risk of chronic liver disease to become protected against HEV infection.

Hepatitis B virus (HBV) accounts for most of the chronic liver diseases (CLDs) in China. Therefore, the safety and immunogenicity data of the hepatitis E vaccine in HBV carriers will be important to the overall vaccine strategy. In a large randomized controlled phase III clinical trial of a recombinant HEV vaccine, HEV239 with the commercial name Hecolin®, demonstrated excellent safety, immunogenicity and efficacy in the general healthy Chinese adult population,[12] which led to the first commercialization of the hepatitis E vaccine.[13] The trial involved 112 604 healthy adults from 11 townships. Among them 14 065
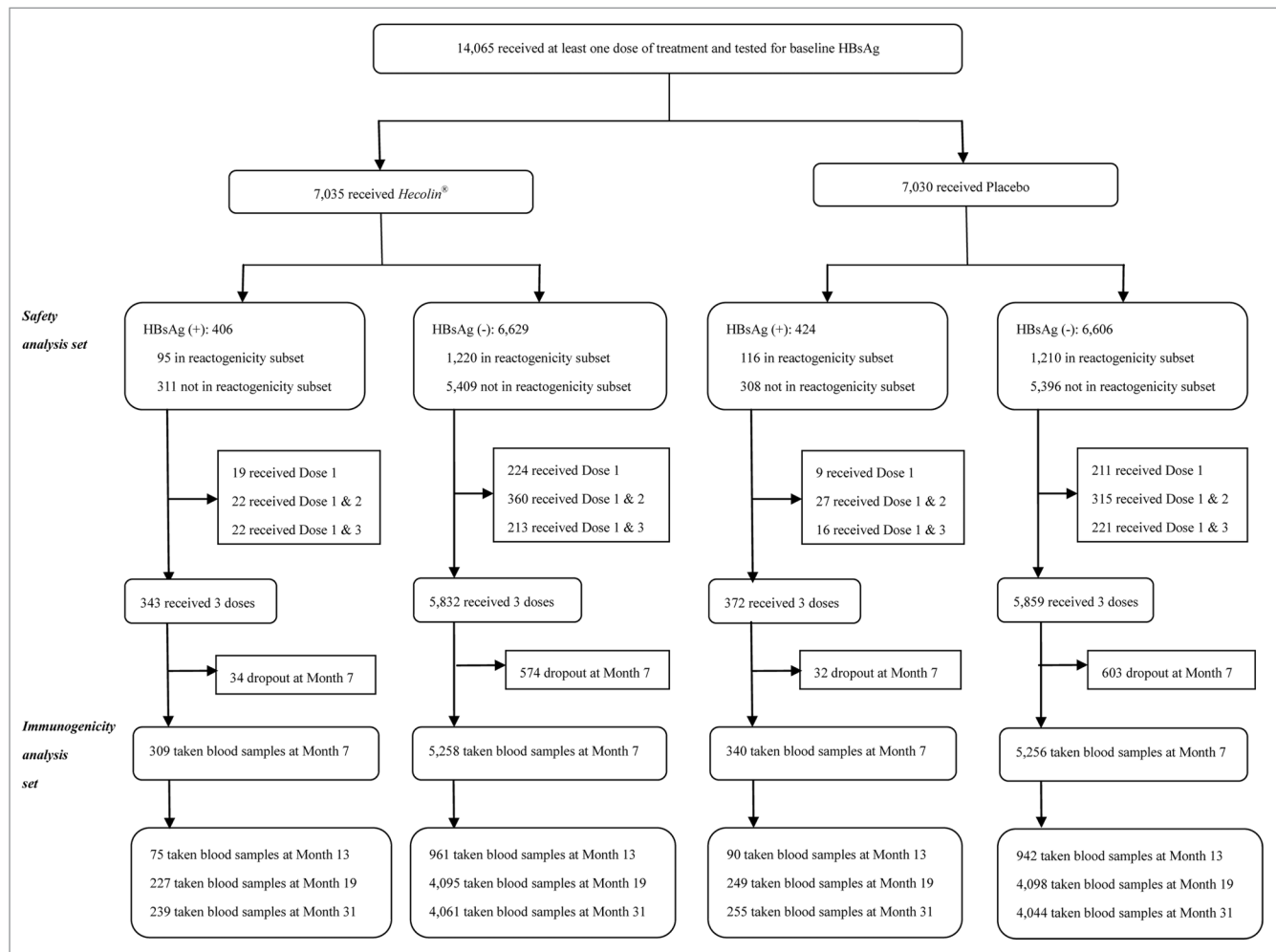
Figure 1. Trial profile.

participants from 2 townships donated their blood samples before and after the vaccination for immunogenicity evaluation. These participants were analyzed according to their baseline HBV status to evaluate the safety and immunogenicity of Hecolin® in HBsAg (+) adults.

Results

Baseline characteristics of the participants

As shown in **Figure 1**, 14 065 participants received at least one dose of Hecolin® or placebo and were tested for HBsAg before vaccination (Safety Analysis Set). Among them, 830 (5.9%) participants were positive for HBsAg. In the Hecolin® group, 309 HBsAg (+) and 5258 HBsAg (-) participants received three vaccine doses and donated their blood samples at month 7 according to the protocol (Immunogenicity Analysis Set). The compliance rates for HBsAg(+) and HBsAg(-) participants were similar for the Hecolin® and placebo groups (p > 0.05). A subset of 2641 participants was selected for active surveillance of adverse events (reactogenicity subset): 95 HBsAg (+) and 1,220 HBsAg (-) participants were in the Hecolin® group, while 116 and 1210 participants were in the placebo group. The baseline characteristics

were generally comparable for the HBsAg (+) and HBsAg (-) populations (**Table 1**).

Safety profile

Most adverse events were mild. Participants in the reactogenicity subset were regularly interviewed by investigators after the receipt of each vaccine dose to assess adverse events (AEs) (**Table 2**). In the reactogenicity subset of the Hecolin® group, 28 HBsAg (+) participants reported solicited adverse events within 72 h. Among them, 11 (11.6%) reported solicited local adverse events, and 21 (22.1%) reported solicited systemic adverse events. The solicited adverse event rates were similar between HBsAg (+) and HBsAg (-) participants in the Hecolin® group (p > 0.05). The HBsAg (+) population in the Hecolin® group reported a higher rate of solicited local AEs than those in the placebo group, which may be due to the higher antigen content of Hecolin®, and in agreement with a previous report.[12] Rates of serious adverse events (SAE) were not significantly different between HBsAg (+) and HBsAg (-) participants in both groups. No vaccine related SAE occurred in any of the subjects during the study.

Immunologic studies

Those participants who received all 3 doses of Hecolin® according to the protocol and had their anti-HEV IgG level

Table 1. Baseline demographic characteristics of the HBsAg (+) and HBsAg (-) participants

	Vaccine		Placebo		P value#
	HBsAg (+)	HBsAg (-)	HBsAg (+)	HBsAg (-)	
All participants (Safety analysis set)	406	6629	424	6606	
Age (SD)	43.95 (10.99)	44.39 (11.31)	45.31 (10.41)	44.36 (11.36)	P1 = 0.4375, P2 = 0.0668
Men (%)	186 (45.81)	2711 (40.90)	199 (46.93)	2739 (41.46)	P1 = 0. 0507, P2 = 0.7461
Positive anti-HEV (prevalence)	183 (45.07)	3158 (48.03)	211 (49.76)	3060 (46.32)	P1 = 0. 3150, P2 = 0.1762
GMC (95%CI) (Wu/mL)*	0.53 (0.44–0.63)	0.54 (0.51–0.56)	0.65 (0.55–0.77)	0.52 (0.50–0.54)	P1 = 0.8915, P2 = 0.0936
Immunogenicity analysis set	309	5258	340	5256	
Age (SD)	44.73 (10.87)	45.25 (10.74)	46.02 (9.93)	45.20 (10.87)	P1 = 0.4102, P2 = 0.1150
Men (%)	135 (43.69)	2036 (38.72)	146 (42.94)	2059 (39.17)	P1 = 0.0819, P2 = 0.8476
Reactogenicity subset	95	1220	116	1210	
Age (SD)	42.92 (11.90)	44.83 (11.17)	44.89 (11.23)	44.95 (11.09)	P1 = 0.1097, P2 = 0.2181
Men (%)	45 (47.37)	478 (39.18)	59 (50.86)	501 (41.40)	P1 = 0.1163, P2 = 0.6135

*GMC: Geometric mean concentration of anti-HEV IgG. #P1-Between Hecolin® Group HBsAg (+) and Hecolin® Group HBsAg (-); P2-Between Hecolin® Group HBsAg (+) and Control Group HBsAg (+).

tested at month 0 and month 7 were included in the immunogenicity analysis set (**Fig. 1 and Table 3**). At month 7, 98.38% and 98.69% of the baseline HBsAg (+) or HBsAg (-) participants showed positive seroconversion, respectively (p = 0.6063). The antibody level of anti-HEV IgG at month 7 in the HBsAg (+) cohort was 19.32 Wu/mL (95%CI, 17.68–21.12), a concentration comparable to that in the HBsAg (-) cohort (19.00 Wu/mL, 95%CI 18.59–19.42) (**Table 3**). The antibody response for each age group was similar for HBsAg (+) or HBsAg (-) participants (**Fig. 2**). Antibody dynamics after vaccination were very similar for HBsAg (+) or HBsAg (-) participants, regardless of their baseline anti-HEV status (**Fig. 3**).

Discussion

In this analysis of the randomized controlled clinical trial of the hepatitis E vaccine Hecolin®, the vaccine was proven to be highly immunogenic and well tolerated for both HBsAg positive participants and persons without HBV infection. This finding is important for the use of the vaccine to prevent the injury of HEV super-infection in HBV carriers.

The data suggest that individuals with chronic liver disease should be prioritized for vaccination to prevent serious damage from HEV infection.[8-11] However, the exclusion of pre-diagnosed CLD patients in enrollment prevented the direct evaluation of the vaccine's safety and immunogenicity in this cohort. There are several other limitations to this study. First, the vaccine efficacy in HBsAg (+) participants cannot be directly calculated due to the low infection rate in the area. Second, the baseline hepatocellular status of these HBsAg (+) participants has not been

tested, and no data of the fluctuation in the hepatocellular status after each dose and after the full vaccination course were available. Thus some subclinical impact of the vaccine may have been overlooked.

Many reports evidence that acute super-infection of the hepatitis virus, hepatitis A virus, hepatitis B virus or hepatitis E virus, in underlying CLD patients resulted in poor prognosis and a high fatality rate.[8-11,14-16] The safety and immunogenicity of the hepatitis A vaccine and the hepatitis B vaccine for CLD patients has been previously demonstrated.[17,18] The results precipitated the recommendation that both vaccines should be administered to CLD patients without the corresponding viral infection. The American Association for the Study of Liver Diseases (AASLD) recommends hepatitis A vaccination for all hepatitis B and hepatitis C virus infected patients, regardless of the stage of their liver disease.[19,20] Furthermore, both the National Institutes of Health (NIH) and AASLD recommend hepatitis B vaccination for chronic hepatitis C patients who are at risk for hepatitis B infection, whereas the Centers for Disease Control and Prevention (CDC) recommend hepatitis B vaccination for individuals with CLD.[21-23] Considering that many "healthy" HBsAg positive individuals suffer from liver damage to some extent,[24] the current finding in "healthy" HBsAg (+) participants paves the way for further studies to assess the benefits of Hecolin® in CLD patients.

In conclusion, this study demonstrated that Hecolin® is safe and immunogenic in HBsAg (+) individuals. HBV carriers have a higher risk of progressing into CLD and cirrhosis. In view of the apparent risks posed by acute HEV infection in patients with CLD, we strongly recommend HBV carriers to be vaccinated with the HEV vaccine.

Table 2. Adverse reactions/events after vaccination with Hecolin® in the HBsAg (+) and HBsAg (-) participants

	Vaccine		Placebo		P value*
	HBsAg (+)	HBsAg (-)	HBsAg (+)	HBsAg (-)	
Participants in the reactogenicity subset	95	1,220	116	1210	
Solicited local adverse events within 72 h					
All	11 (11.58%)	166 (13.61%)	5 (4.31%)	89 (7.36%)	P1 = 0.5770, P2 = 0.0472
≥ Grade 3	0 (0.00%)	2 (0.16%)	0 (0.00%)	0 (0.00%)	P1 = 1.0000, P2 = 1.0000
Solicited systemic adverse events within 72 h					
All	21 (22.11%)	246 (20.16%)	13 (11.21%)	250 (20.66)	P1 = 0.6505, P2 = 0.0322
≥ Grade 3	0 (0.00%)	7 (0.57%)	1 (0.86%)	3 (0.25%)	P1 = 1.0000, P2 = 1.0000
Participants not in the reactogenicity subset	311	5409	308	5396	
Solicited local adverse events within 72 h					
All	6 (1.93%)	106 (1.96%)	2 (0.65%)	53 (0.98%)	P1 = 0.9699, P2 = 0.2859
≥ Grade 3	1 (0.32%)	10 (0.18%)	0 (0.00%)	5 (0.09%)	P1 = 0.4596, P2 = 1.0000
Solicited systemic adverse events within 72 h					
All	4 (1.29%)	96 (1.77%)	3 (0.97%)	91 (1.69%)	P1 = 0.5226, P2 = 1.0000
≥ Grade 3	1 (0.32%)	7 (0.13%)	0 (0.00%)	9 (0.17%)	P1 = 0.3608, P2 = 1.0000
Participants in the whole vaccinated cohort	406	6629	424	6606	
Unsolicited events within 30 d					
All	39 (9.61%)	707 (10.67%)	40 (9.43%)	748 (11.32%)	P1 = 0.5010, P2 = 0.9328
≥ Grade 3	2 (0.49%)	84 (1.27%)	3 (0.71%)	80 (1.21%)	P1 = 0.2407, P2 = 1.0000
SAEs during the period from month 0 to month 31					
All	29 (7.14%)	366 (5.52%)	25 (5.90%)	359 (5.43%)	P1 = 0.1683, P2 = 0.4667

*P1-Between Hecolin® Group HBsAg (+) and Hecolin® Group HBsAg (-); P2-Between Hecolin® Group HBsAg (+) and Control Group HBsAg (+).

Materials and Methods

Participants

The initial efficacy analysis of the trial has been reported previously.[12] In brief, a double-blind, randomized, placebo-controlled trial was conducted from August 2007 through June 2009 in Dongtai City, Jiangsu Province, China.[12] Healthy eligible adults aged 16–65 y without current or previous evidence of chronic liver disease were randomly assigned to receive three doses of the Hecolin® vaccine (containing 30 μg recombinant HEV virus-like particles adsorbed in 0.5 mL of alum adjuvant; Xiamen Innovax) or the placebo hepatitis B vaccine (containing 5 μg recombinant HBsAg in 0.5 mL of alum adjuvant; Beijing

Tiantan Biologic). Written consent was obtained from each participant. Independent Ethics Committee approval was obtained from the Ethics Committee of the Jiangsu Provincial Center for Disease Control and Prevention.

A total of 112 604 adults participated in the trial, enrolled from 11 townships in Dongtai County. Participants living in two townships (Qingdong and Anfeng) who donated their blood samples on month 0 (baseline), month 7 (one month after the third dose), month 19, and month 31 were evaluated for vaccine safety (Safety Analysis Set). A subset of participants from Qingdong township were visited timely at home to observe their adverse events by investigators (Reactogenicity Subset). Other participants were asked to

Table 3. Immunogenicity of Hecolin® in HBsAg (+) and HBsAg (−) participants

Anti-HEV IgG status	HBsAg (+)	HBsAg (−)	P value
Baseline			
Number	309	5258	
No. +ve (rate, %)&	144 (46.60)	2515 (47.83)	0.6740
GMC (95% CI, Wu/ml)*	0.53 (0.43, 0.65)	0.54 (0.51, 0.56)	0.8752
Antibody response			
Seroconversion rate (95% CI, %)			
All	98.38 (96.26,99.47)	98.69 (98.34,98.98)	0.6053
Baseline anti-HEV (+)	96.53 (92.08, 98.86)	97.46 (96.76, 98.03)	0.4207
Baseline anti-HEV (−)	100.00 (97.79, 100.00)	99.82 (99.58, 99.94)	1.0000
GMC on 7 mo (95%CI, Wu/ml)*			
All	19.32 (17.68, 21.12)	19.00 (18.59, 19.42)	0.7271
Baseline anti-HEV (+)	25.33 (22.74, 28.21)	24.71 (24.14, 25.29)	0.6283
Baseline anti-HEV (−)	15.25 (13.42, 17.34)	14.94 (14.44, 15.45)	0.7712

&No of participants who had baseline positive anti-HEV IgG, the ratio in the parenthesis is the positive rate in this group. *GMC, geometric mean concentration.

Figure 2. Anti-HEV IgG levels of HBsAg (+) and HBsAg (−) participants at month 7 by age. Baseline anti-HEV (−) participants who received all 3 doses of Hecolin® according to the protocol were analyzed. Solid black line: HBsAg (+) participants; solid gray lines: HBsAg (−) participants. 95% confidence intervals (CI) are shown. GMC, geometric mean concentration.

Figure 3. Anti-HEV dynamics after Hecolin® vaccination. Participants who received Hecolin® according to the protocol and were tested for anti-HEV at month 0 and month 7 (immunogenicity analysis set) were included. Solid black line: HBsAg (+) participants; dashed gray lines: HBsAg (−) participants. GMC, geometric mean concentration. 95% CI is shown.

record any adverse events on diary cards. Any SAEs were recorded throughout the study for all the participants. Participants who received all three doses of vaccines according to the protocol and who donated paired serum samples at month 0 and month 7 were included in the immunogenicity analysis set.

Laboratory measurements

The anti-HEV IgG were tested and quantified with commercialized assays (Beijing Wantai) and expressed in WHO unit per ml (Wu/ml), as previously described.[12] Those participants whose serum samples at month 7 contained an anti-HEV IgG level four times higher than baseline were labeled as positive for seroconversion. HBsAg was tested with a commercialized assay (Beijing Wantai) according to the product protocol.

Statistical analyses

A chi-square test or a two-sided Fisher's exact test was used to compare the rates between different groups. The independent-sample t-test was used to compare mean age and the geometric mean concentration of anti-HEV between groups. Data analysis was performed with the use of SPSS (Statistical Package for the Social Sciences, version 18.0). All reported P values are two-sided with an α value of 0.05.

Disclosure of Potential Conflicts of Interest

Xiao-Hui Liu and Meng Guo are currently employees of Xiamen Innovax. The other authors declare that they have no conflicts of interest.
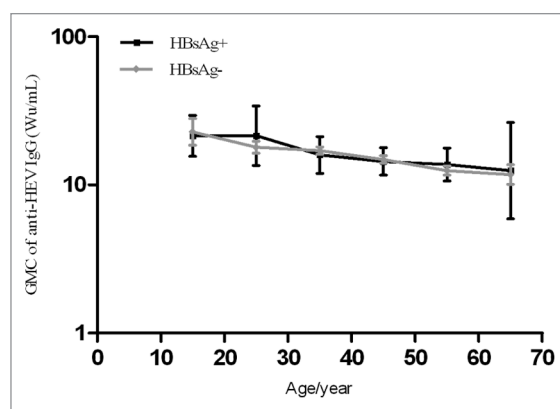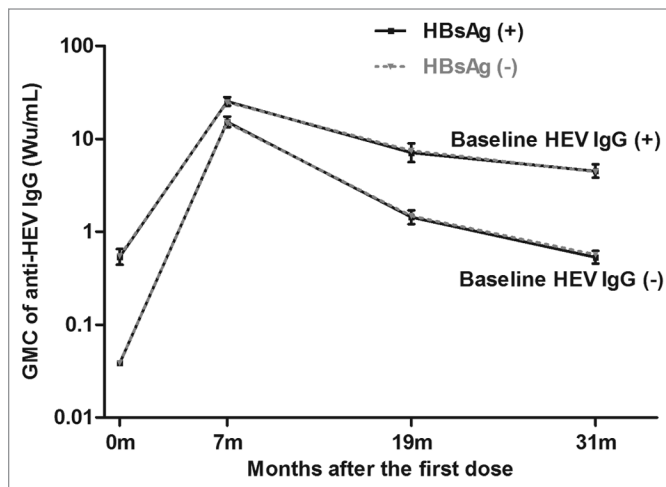
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