Research Article

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Results of a phase III clinical trial with an HBsAg-HBIG immunogenic complex therapeutic vaccine for chronic hepatitis B patients: Experiences and findings

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Background & Aims: Even though various experimental therapeutic approaches for chronic hepatitis B infection have been reported, few of them have been verified by clinical trials. We have developed an antigen-antibody (HBsAg-HBIG) immunogenic complex therapeutic vaccine candidate with alum as adjuvant (YIC), aimed at breaking immune tolerance to HBV by modulating viral antigen processing and presentation. A double-blind, placebo-controlled, phase II B clinical trial of YIC has been reported previously, and herein we present the results of the phase III clinical trial of 450 patients.

Methods: Twelve doses of either YIC or alum alone as placebo were administered randomly to 450 CHB patients and they were followed for 24 weeks after the completion of immunization. The primary end point was HBeAg seroconversion, and the secondary end points were decrease in viral load, improvement of liver function, and histology.

Results: In contrast to the previous phase II B trial using six doses of YIC and alum as placebo, six more injections of YIC or alum resulted in a decrease of the HBeAg seroconversion rate from 21.8% to 14.0% in the YIC group, but an increase from 9% to 21.9% in the alum group. Decrease in serum HBV DNA and normalization of liver function were similar in both groups ($p > 0.05$).

Conclusions: Overstimulation with YIC did not increase but decreased its efficacy due to immune fatigue in hosts. An appropriate immunization protocol should be explored and is crucial for therapeutic vaccination. Multiple injections of alum alone could have stimulated potent inflammatory and innate immune responses contributing to its therapeutic efficacy, and needs further investigation.

Keywords: Phase III; Clinical trial; Therapeutic vaccine; Chronic hepatitis B; Antigen-antibody complex.

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Introduction

Despite the remarkable success of prophylaxis hepatitis B vaccine, there are still around 300 million hepatitis B surface antigen (HBsAg) positive carriers worldwide. Among them, some will progress to chronic hepatitis B, liver cirrhosis, and hepatocellular carcinoma. Despite the fact that antiviral drugs provide inhibitory effects on hepatitis B virus (HBV) replication,

restoration of effective host immune responses is necessary for virus clearance. Currently, several therapeutic vaccines for CHB are in experimental or clinical studies [1–6]. We have developed an antigen-antibody complex (HBsAg complexed with its antibodies) as a therapeutic vaccine candidate for CHB patients. The underlying hypothesis is that CHB patients are immune tolerant to HBV due to the persistence of HBV antigens. The immune system of those patients might be unable to recognize HBV viral antigens as "non-self" and as a consequence cannot generate an effective immune response against the virus. To overcome this defect, the HBsAg is complexed with anti-HBs antibodies at an appropriate ratio. Antigen presenting cells (APCs) will uptake the HbsAg-Ab complex through their Fc receptors. Consequently, the HBsAg will be processed and presented effectively to T cells, triggering an immune response against HBV [7]. This hypothesis was first presented in a pilot study in chronic hepatitis B patients [8], and HBsAg-anti-HBs was shown to enhance T-cell cytotoxicity and decrease serum HBsAg, in transgenic mice [9]. Furthermore, HBsAg-anti-HBs could upregulate HLA-II, CD80, CD86, and CD40 molecules on DCs and improve dendritic and T-cell interactions [10]. In phases IIa and IIb clinical trials, therapeutic efficacy has been shown in CHB patients [11,12]. To further verify the efficacy of YIC, a double-blind, placebo-controlled phase III clinical trial was conducted and the results are presented here.

Materials and methods

Immune complexes and placebo

As reported previously, both the immune complexes (YIC) and placebo used in this study were manufactured by the Beijing Institute of Vaccine and Biological Products with GMP compliance [11]. The recombinant HBsAg (subtype adw2, genotype A2) expressed in yeast, licensed by Merck, was produced by the Beijing Institute of Biological Products, and HBIG was produced by the same institute, and prepared from sera of HBsAg hyperimmunized donors. HBIG has been licensed and used for intramuscular immunization in infants and adults for more than 25 years in China. Each dose of 1 ml YIC consisted of 60 micrograms of yeast-derived recombinant HBsAg complexed with human anti-HBs immunoglobulin (HBIG) at an appropriate ratio following the protocol of production (described in the US patent 6,221,664 B1 and European patent 913157); 0.1% alum was added as adjuvant. The placebo control contained only 0.1% alum without the antigen-antibody complex.

Patients and study design

This double-blind, randomized, placebo-controlled trial was approved by the State Food and Drug Administration (SFDA), China (license number 2002L0038), and was conducted at 21 clinical centers, which were certified by SFDA in China. The trial was registered at WHO International Clinical Trials Registry Platform (ICTRP) (registry number: ChiCTR-TRC-07000019; registry name: A study of the effect of therapeutic vaccine (YIC) for type B hepatitis. URL: http://www.chictr.org/cn/proj/show.aspx?proj=1369). Formal approvals from the ethics committees in 21 evaluation centers were completed, and enrollment of patients started at the end of October 2007. A signed written inform consent for participation in this trial was obtained from each patient prior to enrollment.

The chronic hepatitis B patients enrolled fulfilled the following criteria: 18–65 years of age, HBsAg and HBeAg positive for at least 6 months, anti-HBe negative with HBV viral load >100,000 copies/ml, and serum ALT from two to ten times the upper limit of normal, within four weeks before randomization. Exclusion criteria were co-infection with hepatitis A, C, D, E virus, or HIV; antiviral, hepatotoxic or immunosuppressive drug administration within the 6 preceding months; other causes of liver disease; serious medical or psychiatric illness; hepatic cirrhosis or alpha fetal protein (AFP) >100 ng/ml; abnormal serum creatinine, thrombocyte count, hemoglobin or serum total bilirubin; or pregnancy.

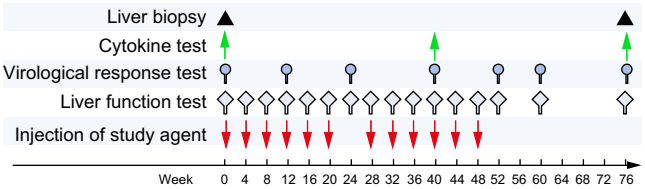

Fig. 1. Diagram of treatment and blood drawn. Twelve intramuscular injections of either YIC or alum were given to each patient. The time for blood sample withdrawal for analysis is indicated in the figure.

All participants were administrated twelve intramuscular injections at 4-week intervals, with an 8-week break between the 6th and 7th injection; the patients were followed-up for 24 weeks after termination of immunization. Due to ethic concerns and for preventing dropout of patients during the 6-month follow-up period without injections, all participants were administered orally two Chinese patented drugs (Silibin Meglumine tablets, and Hugan tablets), which have been reported to protect the liver from injury but have no antiviral effects (Supplementary data A). Serum samples were collected at baseline, week 12, 24, 40, 52, and 76 after initial injection, and screened for HBeAg, anti-HBe, HBV DNA, and alanine aminotransferase (ALT). Liver biopsies were performed at baseline and one month after completion of the follow-up period in a subset of patients selected randomly to assess the histopathological changes (Fig. 1).

All participants were observed for solicited and unsolicited local reactions, systemic symptoms, and severe adverse events through diary cards and follow-up interviews during the entire study period. The causality of adverse events was determined by the chief-clinical investigators.

The study was designed by a chief clinical investigator with collaborators in all centers and was monitored by TigerMed, China, an independent Contract Research Organization (www.tigermed.net). The trial was conducted in accordance with the provisions of the Good Clinical Practice Guidelines.

Randomization and masking

The study was designed in a two-arm fashion. Eligible patients were assigned randomly in recruiting sequence to receive either 60 μg YIC with 0.1% alum as the adjuvant or placebo (0.1% alum) in a ratio of 3:1. A stratified, centralized block randomization method with a block size of eight was applied. Identical labels with computer generated random numbers only were masked to all study agent ampoules. An independent biostatistician was in charge of the processing of randomization using SAS program (SAS Institute Inc., Cary, NC, USA), as well as labeling. The block size and seed numbers were sealed with the randomization list, and were kept at SFDA.

End points

Responses were assessed 4 and 24 weeks after the end of treatment. The primary end point was set as HBeAg seroconversion defined as loss of HBeAg and presence of anti-HBe antibody at the end of treatment. Secondary end points included suppression decrease of HBV DNA, which was defined as a 2 $\log_{10}$ decrease of viral load, normalization of ALT, and improvement of liver histology, at the end of treatment.

Assays for HBV, serological markers, serum cytokines and liver histology

At the end of follow-up, all serum samples were assayed by a central laboratory at Ruijing Hospital, Shanghai, using the same lot of reagents. Sequential samples from one patient were tested on the same day. Abbott EIA AxSYM (Abbott, Abbott Park, IL, USA) was employed for detection of HBsAg, HBeAg, and anti-HBe. HBV DNA was quantified by fluorescent PCR assay (PiJi, Shenzhen Co, China with a detection limit of 500 copies/ml). Architect HBsAg QT assay (Abbott, Abbott Park, IL, USA) was used for serum HBsAg quantification. Routine biochemical and hematological tests were performed at each evaluation center using automated equipment. For histopathological grading, paired liver sections were presented under code to an independent pathologist, using the index of Ishak [13] to evaluate inflammatory and fibrotic changes. Serum samples from all patients who reached the primary end point, and a subset selected randomly from patients who did not reach the primary end point were assayed blindly for IL-2, IL-4,