

Demonstration of safety, immunogenicity and evidences of efficacy of the therapeutic vaccine candidate HeberNasvac and characterization of chronic hepatitis B patient populations

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ABSTRACT

Chronic hepatitis B remains as a major public health problem, with more than 350 million people infected worldwide. Available therapies have limited efficacy and require long-term continuous treatments, further encouraging the development of therapeutic vaccines as a promising approach. In this sense, a new vaccine formulation called HeberNasvac was developed, which is based on the combined administration of the HBV nucleocapsid (HBcAg) and surface (HBsAg) antigens by the intranasal and the subcutaneous routes. In this work, we present some of the achievements of HeberNasvac clinical development studies, particularly summarized data from Phase I and II clinical trials. Altogether, our results demonstrated the good safety and immunogenicity profiles of the HeberNasvac vaccine, providing it as a novel and competitive treatment against chronic hepatitis B. In parallel, the chronic hepatitis B infected populations in Cuba and Bangladesh were characterized, attending to virological, serological, biochemical and histological parameters. This research granted the 2014 Award of the Cuban National Academy of Sciences.

Keywords: hepatitis B, chronic hepatitis B, HeberNasvac, hepatitis B vaccine, clinical trial

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REPORT

RESUMEN

Demostración de la seguridad, inmunogenicidad y evidencias de la eficacia del candidato vacunal HeberNasvac y caracterización de las poblaciones de pacientes con hepatitis B crónica. La hepatitis B crónica es un acuciente problema de salud mundial, con más de 350 millones de personas infectadas. Las terapias disponibles tienen una efectividad limitada e incluyen tratamientos de larga duración, lo que ha estimulado el desarrollo de las vacunas terapéuticas como una alternativa prometedora. En este sentido, se desarrolló una nueva formulación nombrada HeberNasvac, que se basa en la administración combinada de los antígenos de la nucleocápsida (HBcAg) y de superficie (HBsAg) del virus de la hepatitis B (HBV), y de forma simultánea por las vías intranasal y subcutánea. En este trabajo, se presentan algunos de los logros de los estudios de desarrollo clínico de HeberNasvac, en particular un resumen de los datos obtenidos en ensayos clínicos fase I y II. De conjunto, nuestros resultados demuestran el buen perfil de seguridad e inmunogenicidad de la vacuna HeberNasvac, lo que lo convierte en un tratamiento novedoso y competitivo contra la hepatitis B crónica. En paralelo, se pudo caracterizar las poblaciones de pacientes con infección crónica por el HBV en Cuba y Bangladesh, según parámetros virológicos, serológicos, bioquímicos e histológicos. Este trabajo mereció el Premio Anual de la Academia de Ciencias de Cuba para el año 2014.

Palabras clave: hepatitis B, hepatitis B crónica, HeberNasvac, vacuna contra la hepatitis B, ensayo clínico

Introduction

Chronic hepatitis B (CHB) remains as a major public health problem, with more than 350 million people infected worldwide, despite the existence of effective prophylactic vaccines. Chronic infection increases the risk of serious liver diseases such as cirrhosis and hepatocellular carcinoma (HCC). Among the viral factors associated with the severity of chronic infection and increased risk of HCC, the high viral load and the infecting genotypes C and B are the most important ones [1].

Available therapies have limited efficacy and require long-term continuous and expensive treatments, which often lead to the selection of resistant viral variants and rarely eliminate the virus [2]. In this scenario,

immunotherapies have been investigated as a promising approach. Specific immunotherapeutic strategies targeting the induction of CD4⁺ and CD8⁺ T-cell responses and the stimulation of pro-inflammatory cytokines capable of controlling viral replication are under study. Several vaccine formulations have been clinically tested in chronic patients, none of which have clearly demonstrated their efficacy so far [3].

Recently, a new formulation of HBV therapeutic vaccine called HeberNasvac, based on the combination of the HBV core or nucleocapsid (HBcAg) and surface (HBsAg) antigens, was developed at the Center for Genetic Engineering and Biotechnology

1. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology*. 2012;142(6):1264-73.

2. Lok ASF. The maze of treatments for hepatitis B. *N Engl J Med*. 2005;352(26):2743-6.

3. Michel ML, Deng Q, Mancini-Bourguine M. Therapeutic vaccines and immune-based therapies for the treatment of chronic hepatitis B: Perspectives and challenges. *J Hepatol*. 2011;54:1286-96.

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(CIGB). Several preclinical and toxicological studies have been completed in mice and rats, respectively [4, 5]. These studies demonstrated the safety and the high immunogenicity of this vaccine candidate, which is simultaneously administered by the intranasal and subcutaneous routes. Moreover, a group of studies carried out in HBsAg transgenic mice, a hepatitis B chronic carrier model, suggested the potential effect of the vaccination on CHB patients.

So far, several clinical trials were concluded [6, 7]. In this work, the safety and efficacy of HeberNasvac vaccine is demonstrated, further supporting it as a novel and competitive treatment alternative for CHB. In parallel, we collected virological, serological, biochemical and histological evidences characteristic of the CHB affected population in Cuba and Bangladesh.

Results

The HeberNasvac vaccine candidate is safe and immunogenic in healthy volunteers immunized by the intranasal route

First, a double blind, randomized and placebo controlled clinical trial were carried out in 19 healthy male adult volunteers [6]. One group received HeberNasvac using a dose of 50 µg of each antigen (HBsAg and HBcAg), and the other group received placebo (saline phosphate buffer). Both treatments were administered five times using a nasal delivery device. The adverse events (AEs) were actively collected at 1, 6, 12, 24, 48 and 72 h, and at 7 and 30 days after each immunization. In general, the HeberNasvac vaccine candidate was well tolerated and safe. All AEs reported were classified as slight in intensity and auto-limited (self limited). The proportion of AEs observed was similar or lesser than those reported for other commercial products intranasally administered like Nasal-flu, and calcitonin spray [8-10]. Additionally, full seroconversion was attained against HBcAg and a 75 % against the surface antigen. These results are encouraging but also suggested that the intranasal administered dose could be increased to obtain a higher humoral immune response. This clinical trial with HeberNasvac vaccine constituted the first demonstration of seroprotection in healthy adult individuals against hepatitis B virus through the intranasal administration route worldwide.

Evidences of safety, immunogenicity and efficacy of HeberNasvac in CHB patients from Cuba and Bangladesh. Phase I and I/II clinical trials

Afterwards, two clinical trials were done in CHB patients to evaluate the preliminary safety and efficacy in Cuba and Bangladesh. The Cuban study enrolled six patients previously treated with interferon-α, showing detectable levels of viral load at the start of vaccination (unpublished data). The six patients received a vaccination schedule of ten doses each, intranasally. A safe profile was demonstrated in all the patients, with some signals of serological response against the HBeAg and reduced viral load down to levels undetectable by the quantitative system used.

The viral load was stably reduced up to three years after the end of treatment.

The phase I/II trial developed at Bangladesh enrolled 18 treatment-naïve patients [7], the vaccination schedule shown in the figure.

The principal variables of safety (adverse reactions and blood parameters) and efficacy (viral DNA, presence of HBeAg and anti-HBeAg antibodies, and ALT levels) were periodically evaluated during and after treatment, the viral load and biochemical and serological parameters among them. This study further demonstrated the safety profile of HeberNasvac administration in all the treated patients. No dangerous transaminases exacerbations were detected. By the contrary, sustained transaminases normalization was observed, followed by significant reduction of viral load down to undetectable levels in 50 % of the treated patients. Moreover, an increment in the secretion of pro-inflammatory cytokines was observed after the end of the first cycle of treatment. Altogether, these results evidenced that vaccination with HeberNasvac is safe and efficacious, suggesting that this could be an effective therapy against CHB infection. This further supports the realization of other clinical trials. Some relevant features of HeberNasvac's clinical development are summarized in the table.

Characterization of CHB patient populations from Cuba and Bangladesh

During the HeberNasvac clinical development, the phenotypic and genotypic aspects of the CHB patient populations from Cuba [11] and Bangladesh [12] were characterized in a set of studies. The proportion of patients HBeAg-positive and those bearing anti-HBeAg

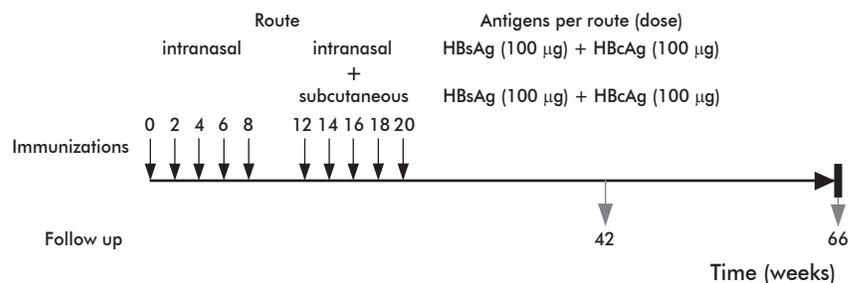


Figure. Immunization schedule with HeberNasvac followed in the phase I/II clinical trial at Bangladesh, in 18 treatment-naïve patients. HBsAg: hepatitis B surface antigen. HBcAg: hepatitis B core antigen.

Table. Data of the clinical profile of the HeberNasvac vaccine candidate against chronic hepatitis B virus (CHB) infection

Parameters	Clinical outcome
Treated individuals	Cuba: 14 (8 healthy and 6 CHB patients) Bangladesh: 18 (CHB patients)
Most frequent adverse events	Systemic: flu-like symptoms Local: Sneezing and rhinorrhea, after intranasal administrations Pain at the injection site after subcutaneous administrations
Main efficacy achievements	Significant and sustained off-treatment reduction of viral load Normalization of transaminases levels
Advantages over established therapies against CHB	Finite treatment Sustained off-treatment virological response Less associated adverse events

4. Aguilar JC, Lobaina Y, Muzio V, Garcia D, Penton E, Iglesias E, *et al.* Development of a nasal vaccine for chronic hepatitis B infection that uses the ability of hepatitis B core antigen to stimulate a strong Th1 response against hepatitis B surface antigen. *Immunol Cell Biol.* 2004;82:539-46.

5. Lobaina Y, Trujillo H, Garcia D, Gambe A, Chacón Y, Blanco A, *et al.* The effect of the parenteral route of administration on the immune response to simultaneous nasal – parenteral immunizations using a new HBV therapeutic vaccine candidate. *Viral Immunol.* 2010;23(5):521-9.

6. Aguilar-Betancourt A, Gonzalez CA, Cinza Z, Cabrera JM, Veliz G, Moreno SR, *et al.* Phase I clinical trial in healthy adults of a nasal vaccine candidate containing recombinant hepatitis B surface and core antigens. *Int J Infect Dis.* 2007;11:394-401.

7. Al-Mahtab M, Akbar SM, Aguilar JC, Uddin H, Khan SI, Rahman S. Therapeutic potential of a combined hepatitis B virus surface and core antigen vaccine in patients with chronic hepatitis B. *Hepatal Int.* 2013;7:981-9.

8. Downs RW, Bell NH, Ettinger MP, Walsh BW, Favus MJ, Mako B, *et al.* Comparison of Alendronate and intranasal Calcitonin for treatment of osteoporosis in postmenopausal women. *J Clin Endocrinol Metab.* 2000;85:1783-8.

9. Nichol KL, Mendelman PM, Mallon KP, Jackson LA, Gorse GJ, Belshe RB, *et al.* Effectiveness of live, attenuated intranasal Influenza virus vaccine in healthy, working adults a randomized controlled trial. *JAMA.* 1999;282(2):137-44.

antibodies was established through virological (viral load and viral genotype) and serological (HBeAg and anti-HBeAg) parameters. In both countries, chronic hepatitis B patients having negative HBeAg serology were predominant, reaching levels of 80 % approximately.

Additionally, different hepatitis B virus genotypes prevailed in both countries. According to the present studies carried out by our group, in Cuba, genotype A is the most frequent, followed by genotype D; while in Bangladesh genotypes C and D are the most common, followed by genotype A at a minor proportion. In Bangladesh, the levels of HBcAg expression in infected hepatocytes were also studied in a group of CHB patients [12]. This variable was correlated with other serological, virological and biochemical variables, resulting an interesting aspect for the therapeutic vaccination. It is known that the induction of an HBcAg-specific T cell response was targeted to hepatocytes carrying viral-derived peptides on their HLA molecules. In general, all these studies increase our knowledge on the hepatitis B chronic patient populations, which are the main targets for the HeberNasvac vaccine treatment.

Relevance of the study

Altogether, the results of the clinical trials shown in this work demonstrate the safety, immunogenicity, and also evidences of efficacy of the HeberNasvac therapeutic vaccine. The use of the intranasal administration route for this vaccine also was endorsed as effective by the clinic data obtained. In general, these results suggest the future successful introduction of this vaccine in the clinical practice. Additionally, the patients' features studied, which were developed while enrolling the volunteers for the different clinical trials in Cuba and Bangladesh, have allowed the in-depth characterization of the HBV chronic infected population in both countries. Similarities were found regarding the proportion of HBeAg negative patients, as well as differences in the main HBV circulating genotypes.

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10. Belshe RB, Mendelman PM, Treanor J, King J, Gruber WC, Piedra P, *et al.* The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine in children. *N Engl J Med.* 1998; 338:1405-12.

11. Loureiro CL, Aguilar JC, Aguiar J, Muzio V, Pentón E, García D, *et al.* HBV Genotypic variability in Cuba. *PLoS ONE.* 2015;10(3):e0118959.

12. Raihan R, Tabassum S, Nessa A, Jahan M, Al Mahtab M, Mohammad C, *et al.* High HBcAg expression in hepatocytes of chronic hepatitis B patients in Bangladesh. *Eurasian J Hepatogastroenterol.* 2012;2(2):63-9.