



Phase I clinical trial in healthy adults of a nasal vaccine candidate containing recombinant hepatitis B surface and core antigens

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Summary

Background: The nasal vaccine candidate (NASVAC), comprising hepatitis B virus (HBV) surface (HBsAg) and core antigens (HBcAg), has been shown to be highly immunogenic in animal models.

Methods: A phase I double-blinded, placebo-controlled randomized clinical trial was carried out in 19 healthy male adults with no serologic markers of immunity/infection to HBV. This study was aimed at exploring the safety and immunogenic profile of nasal co-administration of both HBV recombinant antigens. The trial was performed according to Good Clinical Practice guidelines. Participants ranged in age from 18 to 45 years and were randomly allocated to receive a mixture of 50 µg HBsAg and 50 µg HBcAg or 0.9% physiologic saline solution, as a placebo, via nasal spray in a five-dose schedule at 0, 7, 15, 30, and 60 days. A total volume of 0.5 ml was administered

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in two dosages of 125 μ l per nostril. Adverse events were actively recorded 1 h, 6 h, 12 h, 24 h, 48 h, 72 h, 7 days and 30 days after each dose. Anti-HBs and anti-HBc titers were evaluated using corresponding ELISA kits at days 30 and 90.

Results: The vaccine candidate was safe and well tolerated. Adverse reactions included sneezing (34.1%), rhinorrhea (12.2%), nasal stuffiness (9.8%), palate itching (9.8%), headache (9.8%), and general malaise (7.3%). These reactions were all self-limiting and mild in intensity. No severe or unexpected events were recorded during the trial. The vaccine elicited anti-HBc seroconversion in 100% of subjects as early as day 30 of the immunization schedule, while a seroprotective anti-HBs titer (≥ 10 IU/l) was at a maximum at day 90 (75%). All subjects in the placebo group remained seronegative during the trial.

Conclusion: The HBsAg–HBcAg vaccine candidate was safe, well tolerated and immunogenic in this phase I study in healthy adults. To our knowledge, this is the first demonstration of safety and immunogenicity for a nasal vaccine candidate comprising HBV antigens.

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Introduction

According to the World Health Organization, there were more than 400 million carriers of hepatitis B virus (HBV) worldwide by the year 2000.^{1,2} Among these, approximately 5–10% of adults and 80–90% of children became chronic carriers of the virus. The long-term consequences of chronic carriage are cirrhosis, liver failure and hepatocellular carcinoma.^{3,4} This fact, along with the partial efficacy of antiviral therapies, their cost and side effects, underlines the need for development of more immunogenic hepatitis B vaccines.⁵ This kind of vaccine could be used in specific active immunotherapy in HBV chronically infected patients or chronic carriers.

An impaired T-cell immune response to major HBV antigens is a common scenario in patients with chronic infection. Conversely, patients resolving acute hepatitis, those with chronic infection resolving spontaneously, and patients who control the virus after treatment, display strong polyclonal and multi-specific helper and cytotoxic T-cell responses against HBV nucleocapsid, polymerase and envelope proteins.^{6–9}

Chronic HBV infection has also been associated with functional defects in dendritic cell populations (DC).^{10–12} Additionally, the secretion of HBeAg in saliva of the majority of infected patients has been detected. The negative immunomodulatory effect of the secreted protein in suppressing an inflammatory Th1 response vital for viral clearance has been consistently demonstrated.¹³

Recent developments to boost the weak HBV-specific immune response or to break the non-responsiveness of T-cell immunity in chronic patients include combined prophylactic vaccines, plant-derived vaccines, vaccines administered through novel routes or with novel adjuvants, gene vaccine, and peptide vaccine.^{5,14–24}

The Center for Genetic Engineering & Biotechnology in Havana has developed NASVAC, a novel hepatitis B vaccine candidate for nasal administration comprising HBsAg and HBcAg as vaccine antigens. NASVAC is based on the results of preclinical studies that have demonstrated a good safety and immunogenicity profile.²⁵ Recent advances in the field of devices and methodologies for mucosal immunization further benefit this strategy.²⁶

Nasal administration enables antigens to access very specialized mechanisms for antigen sampling, including antigen uptake by M cells.²⁷ M cells transport antigens from the

luminal surface through a thin cytoplasm to a pocket at the basal surface. M cell pockets enable the interaction of the antigen with the cells of the immune system in a compartment protected from the modulatory effect of systemic immunity. Nasal administration also allows interaction of the antigens with DC in the tonsils, where professional antigen presenting cells (APCs) are organized in a surface network of approximately 500 DC/mm².²⁷

HBcAg has been demonstrated to be the immunodominant antigen at the Th cell and cytotoxic T lymphocytes (CTL) level in patients with self-limited HBV infection and might, therefore, be relevant for virus control.^{24,28–30} Recombinant HBcAg can induce strong HBV core (HBc)-specific Th cell and antibody responses in mice reconstituted with peripheral blood mononuclear cells (PBMCs) from patients with chronic HBV infection.³¹ In vaccine studies, HBcAg has been shown to be a potent immunogen even without adjuvants.^{32,33}

Preclinical studies in mice with the combined HBsAg and HBcAg nasal vaccine candidate showed a high mucosal (nasal) immunogenicity of full length HBcAg and the immunoenhancing activity on co-administered HBsAg.^{25,34–36}

The strong immunogenicity of HBcAg has been attributed to its dual behavior as a T-cell dependent and independent antigen. This is related to the ability of HBcAg to act as a potent B-cell activator, enabling activated B cells to work efficiently as primary APCs.³⁷

Preclinical toxicological studies of the intranasal vaccine candidate were undertaken to determine mucosal irritating potential, acute toxicity, and local tolerance. All the experiments conducted in Sprague–Dawley rats revealed neither clinical adverse signs nor behavioural changes in animals under the studied dose. These results indicated that HBsAg–HBcAg vaccine candidate administered by intranasal route was neither irritating nor induced local damage in the nasal mucosa of the rats.³⁸

In this work we studied the safety and preliminary immunogenicity of nasal co-administration of HBcAg and HBsAg in a phase I clinical trial in healthy adults.

Materials and methods

Vaccine

A single nasal vaccine formulation consisting of a mixture of 50 μ g *Pichia pastoris*-derived recombinant HBsAg subtype

adw2 (CIGB, Havana, Cuba) and 50 µg *Escherichia coli* purified recombinant full length HBcAg (CIGB, Havana, Cuba) was used (Genbank accession number X02763). HBsAg was produced as a 22 nm particle to more than 95% purity at the CIGB production facilities as a component of the commercial anti-HBV vaccine, Heberbiovac-HB[®]. The HBsAg antigen for this vaccine only comprises the S protein, expressed and purified as a non-glycosylated form. HBcAg was purified from *E. coli* strain W3110, which had been transformed previously with a plasmid containing the entire core antigen gene under the control of a tryptophan promoter.^{35,36,39} The resulting HBcAg had a purity superior to 95% and a size of 28 nm as characterized by electron microscopy (EM) analysis.²⁵ Further antigenicity studies were conducted to characterize this particle.³⁴ The vaccine candidate was diluted in sterile phosphate buffer without any adjuvant. The mixture was a sterile, aqueous and transparent liquid. Each vial contained 0.6 ml of the vaccine formulation. Sterile physiologic saline solution at 0.9% was used as placebo. All study products were stored in borosilicate 2R bulbs at 4 °C until use. Nasal spray devices (Accuspray[®], Becton-Dickinson) were filled on the day of the inoculation under sterile conditions.

Study population

Subjects were healthy male adults between 18 and 45 years of age. Volunteers were recruited into the study after obtaining written informed consent. Individuals were eligible if they had no history of hepatitis B infection or immunization with hepatitis B vaccine, and were negative for HBsAg and antibody tests against HBsAg and HBcAg. Individuals were excluded from enrollment if they had clinically significant acute or chronic diseases, immunosuppressive disorders or medication, prior inoculation of any other nasal drug, behavioral risk factors that might have resulted in recent exposure to hepatitis B virus, treatment with blood products or immunoglobulin within 6 months of study entry, a history of sensitivity to any component of the study vaccines, or had abnormalities in blood screening regarding chemical and basic hematology.

Study design, randomization, blinding and immunization procedures

The study was designed as a single center, randomized placebo-controlled double-blinded study in 19 healthy male adult volunteers. Written informed consent was obtained from all participants prior to any study procedure; the study was approved by the Research Ethics Board of the National Center for Toxicology (CENATOX) and "Carlos J. Finlay" Hospital (Havana, Cuba). All the participants were randomly allocated in a ratio of 1:1 to vaccine or placebo group in a balanced format using a computer-generated list of random numbers. The vaccine was administered using a nasal spray device (Accuspray[®], Becton-Dickinson). Depending on the group to which each subject was assigned, the volunteer received either NASVAC or placebo delivered in a fixed volume of 125 µl in order to increase the residence time of the drug over the mucosa and to avoid anterior and posterior dripping of the vaccine formulation in the nose or digestive tract. The study nurse sprayed the formulation in each nostril with the participant's head tilted backwards which allowed expiration

through the mouth. Two applications per nostril were given 15 minutes apart. All subjects received a total dose of 0.5 ml. Participants allocated to placebo received the same volume of 0.9% sterile saline solution. Although there was no possibility that study staff or volunteers could detect differences between vaccine formulation and placebo, study staff who administered the vaccine were not involved in collection of any clinical data. The recording of adverse events was made actively, and always by a trained physician specializing in internal medicine, toxicology or otorhinolaryngology. Neither the study nurse who performed the inoculations at the time of immunization, nor the physicians or volunteers were aware of the vaccine allocation. All aspects of the trial were closely assessed by a medical monitor from the sponsor institution. Five doses of the vaccine were given via nasal spray at days 0, 7, 15, 30, and 60.

Clinical and serological monitoring

Participants were kept under observation at the hospital for 48 hours on administration of the first and the second vaccine doses. Adverse events were actively recorded by study personnel before and 1 hour after each immunization for any immediate adverse events as well as at 6 h, 12 h, 24 h, 48 h, 72 h, and 7 days post-immunization. The observation period concluded at day 90 of the immunization schedule. Following administration of the third vaccine dose the trial was carried out on an outpatient basis but adverse events were monitored over the same time intervals with the exception of the observations at 6 h and 12 h post-immunization. Solicited adverse events included anterior and posterior nasal dripping, nasal stuffiness or congestion, sneezing, nasal itching, palate itching, anosmia, nasal ulceration, and nasal bleeding. Solicited systemic adverse events included fever, chills, headache, muscle aches, decreased appetite, nausea, vomiting, diarrhea, and cutaneous rash. Information on any other symptoms, the use of non-steroid anti-inflammatory analgesics, non-planned medical consultations or doctor's visits, and hospitalizations were also collected and categorized by body system. Adverse events were defined as absent, mild (symptom present but not bothersome, a nuisance at most), moderate (symptom bothersome, discomfort enough to cause interference with usual activity, frequent and annoying, may require medical attention), or severe (symptoms very distressing, interference with normal functioning, may require medical attention). Serious adverse events were defined as events that were fatal or life-threatening, that caused a prolonged hospitalization, that resulted in a significant, persistent, or permanent disability, or that required intervention to prevent permanent impairment or damage. In addition to self-reporting of nasal adverse reactions, a single otorhinolaryngologist performed periodical examinations with visualization of the nasal mucosa, septum and mucosal blood vessel status after the administration of each dose of the nasal vaccine. Furthermore, participants were instructed to measure their axillary temperature and to recognize any local or systemic adverse events.

Blood, saliva and nasal wash samples were collected at baseline, 30 days and 90 days following the first inoculation. Blood was obtained by venipuncture for measurement of serum clinical chemistry and basic hematological parameters. The total serum antibody response to HBsAg and

HBcAg was measured by commercial ultramicroanalytic enzyme linked immunosorbent assay (anti-HBs/anti-HBc UMEELISA[®], Immunoassay Center, Havana, Cuba).^{40,41}

Briefly, anti-HBs UMEELISA⁴⁰ is an enzyme-linked immunosorbent assay (ELISA) adapted to small volumes of samples and reagents (10 μ l), based on the principle of neutralization using the same typical sandwich as in other commercial anti-HBs ELISA kits. Samples are pre-incubated with natural HBsAg (subtype *adw2*) obtained from serum of a chronic HBV infected patient with high HBsAg titer. Anti-HBs in sera block antigenic determinants on the HBsAg. Recombinant HBsAg reacts with solid phase monoclonal antibodies. The binding of HBsAg is evidenced by the successive reaction of the conjugate anti-HBs—alkaline phosphatase and a fluorescent substrate. The reduction of the fluorescent signal will be proportional to the concentration of anti-HBs in the sample.

Anti-HBc UMEELISA⁴¹ is a competitive sequential immunoenzymatic assay for qualitative detection of total anti-HBc antibodies in human sera. Briefly, UMEELISA plates (10 μ l per well) coated with commercial recombinant HBcAg (Bio-kit, Barcelona, Spain) are incubated with samples. Solid phase antigen will react with antibodies from serum. A rabbit anti-HBc—alkaline phosphatase conjugate is added, reacting with remaining determinants. After the addition of a fluorogenic substrate, the intensity of the emitted fluorescence will be inversely proportional to the anti-HBc in the sample.

All blood samples were evaluated in duplicate in a blinded fashion on code-labeled, matched pre- and post-immunization sera. Only anti-HBs antibodies were measured in saliva and nasal wash samples. All anti-HBs antibody levels were expressed as international units per liter (IU/l) according to the World Health Organization standard. The criterion for protection was defined as concentration of anti-HBs equal to or higher than 10 IU/l. Thus, the subjects were classified as non-responders (anti-HBs <10 IU/l), low-responders (anti-HBs 10–100 IU/l), and high-responders (anti-HBs >100 IU/l).^{42,43} Sera with undetectable anti-HBs antibody were assigned arbitrarily a value of 0.2 IU/l for analysis.

Anti-HBc antibody was expressed as a positive or negative result. On completion of the study, the recombinant Heberbiovac HB[®] (CIGB, Havana, Cuba) licensed hepatitis B vaccine was provided to participants who had not achieved antibody levels ≥ 100 IU/l.

Data analysis and statistical considerations

Baseline comparability of the groups was assessed by Fisher's exact test for proportions and the *t*-test for continuous variables. Adverse events were tabulated by time (day) and by severity (mild, moderate, and severe). Clinically significant events were defined as an axillary temperature ≥ 38.0 °C and any other symptom graded as moderate or severe. Severe reactions were defined as axillary temperature ≥ 39 °C and any symptom graded as severe. Local site reactions were combined to give an 'any local' reaction category and all other reactions combined to give an 'any general' reaction category. The proportion of subjects having an adverse reaction was estimated by treatment group, observation period, and severity. Binomial distribution point estimates and 95% confidence intervals were used to estimate each rate; percentages were compared by Fisher's exact test. A *p* value <0.05 was considered statistically significant; no adjustments were made for

multiple comparisons. Geometric mean anti-HBs antibody levels and 95% confidence intervals were estimated pre- and post-immunization.

The primary outcome of the study was the proportion of subjects reporting specific post-inoculation adverse reactions. The secondary outcomes were: the proportion of subjects showing antibody levels against HBsAg ≥ 10 IU/l (seroprotection percentage), anti-HBc seroconversion after immunization, and the geometric mean anti-HBs antibody level. As this was a phase I, first-in-human clinical trial, no formal hypothesis testing was planned and no formal sample size calculation was performed; however, each treatment group sample size (nine participants as minimum) ensured that the probability of detecting at least one adverse event in the group was 0.73, provided that the true adverse event rate exceeded 15%.

Results

Demographics

A total of 43 subjects signed a written informed consent and underwent pre-study screening; reasons for 'screen failures' included persistently abnormal baseline biochemistry or hematology tests (4), pre-existing antibody against hepatitis B virus (anti-HBs or anti-HBc antibodies) (17), and inability to contact after the screening visit (3). The remaining 19 participants were randomized and received study drug. The mean age of participants was 29.7 years (range 18–45 years). There were no differences in the age, weight and height between vaccine and placebo group. Individuals of white race predominated during the trial. Nine participants received the nasal vaccine candidate and 10 the placebo solution. All but two participants completed the study; one subject in the vaccine group and one in the placebo group withdrew voluntarily from the study prior to the fourth immunization. Withdrawal from the trial was not related to any adverse event in both cases.

Safety and clinical adverse events

Exposure to the nasal formulation was evaluated during a 90-day period following administration of the first vaccine dose at time 0. No abnormalities in clinical chemistries and hematology values were found in participants during the study. A total of 77 adverse events were recorded during 90 applied doses (85.5%). More than 97% of adverse events were mild in intensity and 98.7% of them were requested on the data collection sheets. The 77 recorded adverse events were reported by 15 participants of the 19 included in the statistical analysis. There was no difference in the proportion of subjects reporting adverse events between study and control group. The reporting of adverse events within the first hour after vaccine administration was infrequent. Adverse events were mostly reported during the first and second dose and between 24 h and 72 h following inoculation. The recording of local adverse events predominated over systemic reactions (71.4%). The vaccine group reported the highest frequency of local adverse events (58.2%), whereas systemic adverse effects predominated in the placebo group (59.1%). The most commonly reported nasal adverse events in the study group included sneezing (34.1%), rhinorrhea (12.2%),

Table 1 Frequency of each type of adverse event with respect to the total number of applied doses per study group

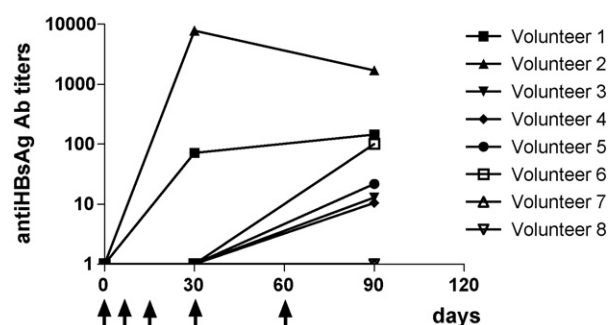
Adverse event/ group	Vaccine candidate	Placebo	Total
Total of applied doses	42	48	90
Requested adverse events			
Sneezing	14 (18.2%)	4 (5.2%)	18 (23.4%)
Rhinorrhea	5 (6.5%)	3 (3.9%)	8 (10.4%)
Nasal itching	1 (1.3%)	8 (10.4%)	9 (11.7%)
Nasal stuffiness	4 (5.2%)	2 (2.6%)	6 (7.8%)
Local pain	0	1 (1.3%)	1 (1.3%)
Epistaxis	2 (2.6%)	0	2 (2.6%)
Palate itching	4 (5.2%)	0	4 (5.2%)
Anosmia	0	2 (2.6%)	2 (2.6%)
Odynophagia	2 (2.6%)	2 (2.6%)	4 (5.2%)
Local edema	0	1 (1.3%)	1 (1.3%)
Headache	4 (5.2%)	4 (5.2%)	8 (10.4%)
Febricula	1 (1.3%)	2 (2.6%)	3 (3.9%)
Asthenia	0	5 (6.5%)	5 (6.5%)
General malaise	3 (3.9%)	2 (2.6%)	5 (6.5%)
Unsolicited adverse events			
Vasovagal syncope	1 (1.3%)	0	1 (1.3%)
Total	41 (53.2%)	36 (46.8%)	77 (100%)

Source: data collection sheets.

nasal stuffiness (9.8%), palate itching (9.8%), headache (9.8%), general malaise (7.3%), and epistaxis (4.9%). Only the frequency of sneezing was significantly higher for the vaccine recipients ($p = 0.0038$). All reactions were self-limiting, resolving within 72 h after inoculation, and were mild. No development of mucosal alterations or transient nasal conditions occurred during the study. No cases of fever were registered. Only two moderate adverse events were reported during the trial corresponding to headache; both reports came from the placebo group. No unexpected or severe adverse event was recorded during the trial (Table 1).

Antibody response

At baseline, all participants were seronegative for anti-HBs and anti-HBc antibodies (Table 2). No volunteers inoculated with the placebo developed antibodies against HBsAg or HBcAg. A total of two (25%) of the eight recipients of the nasal vaccine candidate developed seroprotective antibody titers against HBsAg and 100% showed seroconversion against

**Figure 1** Kinetics of the anti-HBsAg antibody response in healthy adults nasally immunized with HBcAg–HBsAg vaccine candidate. Vaccine schedule: 0, 7, 15, 30, and 60 days. Blood samples were collected from subjects at days 30, 60, and 90. Vertical arrows: nasal vaccine inoculations.

HBcAg at day 30 of the immunization schedule. In two seroprotected vaccine recipients at day 30, one was considered a hyper-responder (>100 IU/l) and the other developed anti-HBs titers around 70 IU/l. One recipient of the nasal vaccine and one from the placebo group withdrew from the study prior to the fourth dose and sera were not available for testing.

At day 90, 75% (6/8) of vaccine recipients achieved anti-HBs protective levels (Figure 1). At this time 37.5% of seroprotected subjects were considered hyper-responders and a similar proportion hypo-responders. The geometric mean antibody titer at this time was 64.8 IU/l. All the volunteers in the study group remained positive for anti-HBc antibodies. Thus, most of the participants needed a fourth or a fifth dose to achieve protective anti-HBs antibody levels.

Discussion

The results of this phase I study indicate that the nasal vaccine candidate formulated with 50 μ g HBsAg mixed with 50 μ g HBcAg was well tolerated, safe and immunogenic in healthy adults. Local site adverse events were mild, self-limiting, and disappeared within 72 hours following inoculation. Adverse events did not increase in frequency with the administration of successive vaccine doses. Local adverse events were reported more frequently in participants inoculated with the nasal vaccine candidate, whereas systemic adverse events were more frequently reported among individuals of the placebo group. Sneezing, palate itching, rhinorrhea, nasal stuffiness and epistaxis were the most frequent adverse events reported in the vaccine group, with approximately 2.6–13% excess over the frequency in the

Table 2 Antibody response to HBcAg–HBsAg vaccine candidate in healthy volunteers immunized by the nasal route with 50 μ g HBcAg non-covalently linked to 50 μ g HBsAg following the schedule 0, 7, 15, 30, and 60 days

Group/time	Vaccine candidate			Placebo		
	Day 0	Day 30	Day 90	Day 0	Day 30	Day 90
N	9	8	8	10	9	9
Anti-HBcAg seroconversion %	—	8 (100%)	8 (100%)	—	—	—
Anti-HBs seroprotection % (anti-HBs ≥ 10 IU/l)	—	2 (25%)	6 (75%)	—	—	—

Source: data collection sheets.

placebo group. No subjects withdrew from the trial because of an adverse event. There were also no clinically apparent abnormalities visualized in vaccine recipients according to otorhinolaryngologist examination.

The rates of local adverse events in this study were similar to those reported by other investigators with licensed nasal flu vaccine using similar surveillance methods.⁴⁴ Concurrent comparison with other licensed nasal vaccine (e.g. influenza, nasal flu) would be informative as this vaccine has an acceptable local adverse event profile under routine use.

In this study, 100% of the participants inoculated with the nasal vaccine candidate demonstrated seroconversion for anti-HBc antibodies and 25% showed protective levels of anti-HBs only 30 days after the first inoculation, using an immunization interval protocol of 0, 7, 15, 30, and 60 days. All sera were available for screening at day 30. Thirty days after the fifth vaccine dose the seroprotection rate increased to 75%. By comparison, licensed HBV is reported to elicit a seroprotective response in up to 20% of healthy young adults 30 days after the first dose injection and up to 71% in recipients one month after the second dose.^{45–47} In this sense, although three doses were administered by day 30 to get 25% seroprotection, we consider our results to be very encouraging taking into consideration that the formulation and devices still need to be improved.

The dose of 50 µg of HBcAg was enough to elicit seroconversion in 100% of vaccine recipients as early as the third dose; however five vaccine doses were needed to reach 75% seroprotection with respect to anti-HBs titers of more than 10 IU/l. Although anti-HBc quantification was not possible, the strong immunogenicity of this antigen is clearly evident and corroborates previous findings in experiments in mice with full-length recombinant HBcAg. The strong mucosal immunogenicity is also likely to be related to its particulate nature as has been reported previously for other particulate antigens.⁴⁸ The presence of nucleic acids bound to the C-terminal region of the HBcAg molecule, as revealed by EM analysis, could also contribute to the strong immunogenicity of HBcAg. *E. coli* nucleic acids are co-purified with recombinant HBcAg particles as a nucleoprotein in trace amounts.²⁵

Similarly, we have confirmed the detection of a positive response in a nasal wash and saliva from one hyper-responder volunteer generating more than 10 IU/l in such samples. However, our methods for the detection of IgA should be improved in terms of sensitivity. Similarly, further assays are required for the detection of cellular responses against HBsAg and HBcAg, considering the potential therapeutic use of the present formulation. Phase II clinical studies should address this issue.

This study clearly demonstrates that nasal HBsAg–HBcAg vaccine candidate was well tolerated and reasonably immunogenic for the dose of HBsAg used and the stage of the trial (phase I). However, further studies should address the impact of HBcAg immunomodulation on HBsAg immunogenicity. Pre-clinical mouse studies have fully demonstrated the immunopotentiating activity of HBcAg over nasally/parenteral administered HBsAg in mice and rabbits, with induction of a Th1 type of cytokine response in mice.^{25,34–36} The addition of HBcAg to HBsAg significantly increases the rates of seroprotection over that achieved by nasal immunization with HBsAg alone or mixed with other mucosal adjuvant (e.g. acemannan). Whether the enhancement observed in the

animal studies is relevant to the clinical situation should be confirmed in further immunogenicity studies.

Despite the limitations explained above, the nasal vaccine candidate tested was well tolerated and induced serum antibody responses against both antigens. Rates of adverse events were not substantially different than those reported in recipients of other nasal vaccines currently licensed by the Food and Drug Administration in the USA (e.g. nasal-flu) or other well-known nasally-administered drugs of protein nature (e.g. Miacalcin[®] nasal spray, calcitonin-salmon, Novartis).^{44,49}

Induction of immunity against HBcAg and HBsAg exploiting the mucosal immune system could be an effective approach in the enhancement of antiviral immunogenicity in populations known to be hypo- or non-responders to the standard parenteral HBV vaccine, such as older individuals, renal dialysis patients, and immune compromised hosts, or in populations that are difficult to access.^{50–52} Furthermore, local and systemic immunity elicited by the nasal route of immunization could be useful to overcome the well-documented state of unresponsiveness characteristic of HBV chronic infection and the carrier state.

The results of this phase I study support further phase II studies with HBsAg–HBcAg combined formulations in healthy adults in order to optimize the dose, schedule, formulation, and device. Also, chronic HBV infected patients and carriers of the virus, as well as hypo- and non-responders to licensed hepatitis B vaccines should be studied. To our knowledge, this is the first report in humans of the administration of HBV antigens by nasal route, showing induction of immunity against HBV infection.

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