

Research Article

HeberNasvac, a Nasal and Sublingual Vaccine Formulation with Interferons Inducing Capacity.

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Abstract

The combination of nasal and sublingual administration of HeberNasvac to subjects and patients during the Covid19 pandemic, demonstrated safety and local and systemic innate immune-stimulatory properties. With the aim of compare different mucosal administration routes and methods, an open-label phase II clinical trial was carried out with 40 elderly volunteers randomized into 4 groups. It was studied the expression of α , β , and γ interferons, and also, the safety of HeberNasvac. It was confirmed that HeberNasvac was safe in the 4 treatment groups. There was a generalized stimulation of the interferon genes expressions in the PBMC of most volunteers, in samples taken on days 8 and 15 after immunization, compared with day 0 (not treated). There were no significant differences between the 4 treatments, but interferon gene expression was greater on day 8 in the majority of the groups. These results shown the ability of the mucosal administration of HeberNasvac (inclusive the sublingual route alone) to induce an interferon expression stimulation in the systemic compartment of elderly volunteers and suggest its potential use in epidemics of infectious respiratory diseases. The study was indexed at the Cuban Public Registry for Clinical Trials with the number RPCEC00000326-Sp.

Keywords: HeberNasvac, sublingual immunization route, nasal immunization route, early therapy, post exposure prophylaxis, acute respiratory diseases.

INTRODUCTION

Since 2003 it was proposed that innate immune-activating compounds based on conserved microbial components (recognized by toll-like molecules and other receptors) could be used as broad-spectra anti-infectious agents [Hackett, 2003]. This is in line with the fact that innate immunity evasion is a common event for respiratory and RNA viruses [Kikkert, 2020; Ouyang et al, 2022; Nelemans and Kikkert, 2019; Thorne et al, 2022], highlighting the rationale of using innate immune activation for the post exposure prophylaxis and early therapy of a broad spectrum of viral infections. Thus, post-exposure prophylaxis approaches as early therapies involving

innate immune stimulation are plausible actions to prevent or ameliorate respiratory infections like that of SARSCoV2 coronavirus infection [Sheahan et al, 2008; Zhao et al, 2012; Kumaki et al, 2017; Marx et al, 2022; Tamir et al, 2022].

HeberNasvac, a therapeutic vaccine for Chronic Hepatitis B (CHB), administered by the intranasal (IN) and subcutaneous (SC) routes in several clinical trials have been demonstrated its safety and efficacy [reviewed Aguilar et al, 2022a], and also may be used as a potential innate immune stimulator, as a second indication, and/or a dedicated formulation [reviewed Aguilar et al, 2022b].

The innate immune agonist effect of HeberNasvac depends on the chemical composition of its antigens since both are

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virus-like particles (VLPs). HBcAg is a nucleoprotein and the HBsAg is a lipoprotein, both with innate immunity ligands. Nucleic acid composition associated to HBcAg moiety was of 20% RNA and 1% DNA from *E coli* which could trigger an agonist/receptor activating effect on several TLRs [reviewed Aguilar et al, 2022a, Aguilar et al, 2022b]. Some of these TLRs are considered relevant for Covid19 immuno-pathogenesis. For example, TLR3 stimulation in animal models has been associated to protection from lethal-challenges of SARS and Influenza A infection [Zhao et al, 2012; Kumaki et al, 2017].

Although SARSCoV2 coronavirus can infect any age group, it produces worse clinical conditions in the elderly, leading to complications and death, thus enhancing morbidity and mortality [Zhang et al, 2022].

Within the context of the Covid19 pandemic, an exploratory clinical trial in patients treated with HeberNasvac compared to untreated was carried out in elderly (60 years or older), at the risk of a SARSCoV2 infection. The stimulation of the expression of TLR3, TLR7 and TLR8 genes in oropharyngeal swabs was detected together with the increase of the HLA-DR expression levels in PBMC, indicating that the immune system is better prepared to resist a viral aggression. HeberNasvac was administered through a nasal spray (0-7-14 days) and sublingual (SL) drops daily, from day 0 to day 14 [Fleites et al, 2021]. In a larger study in elderly volunteers, also evaluating a treatment schedule similar to Fleites et al, 2021, HeberNasvac promoted the gene expression in blood of multiple IFN-stimulated genes (OAS1, ISG15, ISG20, STAT1, STAT3) after 5 days of the immunizations when was compared with the untreated [Aguilar et al, 2024].

This study is aimed at confirming the safety and the capacity of HeberNasvac VLPs to induce innate immunity. Results here show that the induction of innate immunity was demonstrated by the stimulations of the expression of several interferons genes in PBMC of elderly volunteers after the mucosal administration of HeberNasvac, combining nasal and sublingual routes, and also nasal or sublingual routes each alone.

MATERIAL AND METHODS

Product

HeberNasvac is a therapeutic vaccine for chronic hepatitis B administered by the IN and SC routes [reviewed Aguilar et al, 2022a; Aguilar et al, 2022b]. The vaccine comprises the HBsAg antigen and the HBcAg antigen of the Hepatitis B virus (HBV, Gen A, Adw2) produced by recombinant DNA technology as virus-like particles. Specifically, at the Center for Genetic Engineering (CIGB) the HBcAg is produced in *E.coli*, and has unique characteristics: a higher proportion of RNA to protein, compared to other HBcAg reported in the literature, as demonstrated by deep sequencing analysis, and further

supporting its immunomodulatory effect [reviewed Aguilar et al, 2022a]. HeberNasvac was released following strict quality specifications, obtained under a GCP production system, and it was registered for the treatment of CHB in Cuba. It contains 100 µg of each antigen in a final volume of 1.0 mL in saline-phosphate buffer, pH 7.0. No other additives, preservatives or stabilizers are included. The antigens and the formulation were produced and released under GMP conditions at the production facilities of CIGB.

Clinical Trial

A randomized, open and prospective Phase I/II clinical trial named “Virma”, was carried out with HeberNasvac administered by different routes and administration methods to assess immunogenicity against major interferon gene markers after 8 and 15 days from the start of the study, and also confirm safety. The study included 40 healthy volunteers of both sexes, of 60 years of age, or older, which also are not hepatitis B chronic patients. The study was indexed at the Cuban Public Registry for Clinical Trials (RPCEC) with the number RPCEC00000326-Sp. The trial started after the approval of the protocol by the ethical committee of the CIGB and with the written authorization of the national regulatory authority (CECMED). The design and conduction of the study complied with the principles of the Helsinki Declaration. All volunteers were enrolled after signing the informed consent. The working hypothesis was that HeberNasvac only administered by the mucosal route, is able to stimulate innate immunity in blood (PBMC) at the level of the interferon gene markers in at least the positive control treatments (here group 1 or G1) [Fleites et al, 2021; Aguilar et al, 2022; Aguilar et al, 2024], keeping its demonstrated safety in several clinical trials [Fleites et al, 2021; reviewed Aguilar et al 2022a; Aguilar et al 2024].

Design of the study

The study included elderly willing to participate as confirmed by their signed informed consent. The exclusion criteria encompassed the presence of diabetes, cardiovascular disease, kidney or autoimmune diseases, oncological diseases, high levels of atherosclerosis, obesity and chronic inflammatory disorders. The persons infected with SARSCoV2 within 14 days before the recruitment, were also excluded. Furthermore, the exclusion criteria comprised subjects taking immune-suppressors, under chemotherapy or using any of the products under study 30 days before the recruitment. Subjects with fever (temperature $\geq 38^{\circ}\text{C}$) and those that had been submitted to throat surgery were not included. Finally, patients with liver cirrhosis, transplants, allergy to any of the ingredients of the vaccine, or having any problem that would prevent them from an appropriate follow-up, were also excluded.

The study encompassed 4 groups of 10 volunteers each. Group 1 (G1) i.e. the positive control group, was submitted to the previously reported treatment (IN spray on days 0, 7 and 14 and SL drops from day 0 to 14) [Fleites et al, 2021; Aguiar et al, 2022; Aguiar et al, 2024]. Group 2 (G2) received IN drops on days 0, 7 and 14 and SL drops from day 0 to 14. Group 3 (G3) received nasal drops alone (days 0, 7 and 14) and group 4 (G4) was treated with SL drops alone (from day 0 to 14). It was not possible to have a negative control group because all the elderly wanted to be treated, so the untreated time was always the same for the four groups, the day 0 or time 0 (T0) before the administration of the products.

Evaluations

Safety and adverse events

The study evaluated clinical adverse events occurring during the first hour and the first 24 hours after administering the product. The type of adverse event, their duration, intensity, as well as the procedure followed and the outcomes after each immunization are described.

Innate immunity assessment

Samples

The blood samples for the innate immunity tests were obtained before administering the treatments (day 0), and on days 8 and 15 after starting the treatments. Although there were 10 subjects per group for the safety study, the blood samples were extracted from only three or four available volunteers in each group according to the feasibility of the elderly and the laboratory. Fourteen blood samples were taken by venipuncture from the median cubital vein, and consecutive numbers were assigned to these samples designated for qPCR analysis (from 1 to 15, sample number 6 was discarded: G1: samples 1, 2 and 3/ G2: 4, 5, 7, 8 / G3: 9, 10, 11/ G4: 12, 13, 14, 15). They were then distributed in CPT-heparin tubes for peripheral blood mononuclear cell (PBMC) isolation (BD 362753).

RNA Purification and cDNA synthesis

PBMC derived RNA was extracted using the "RNeasy Mini Kit (250)" (Qiagen, Gilden, Germany), following the manufacturer's instructions. RNA was quantified using a NanoDrop spectrophotometer (Thermo-Scientific, Waltham, Massachusetts, United States). In the reverse transcription (RT) reaction for obtaining cDNA we followed the manufacturer's instructions from the Quantitect Reverse Transcription Kit (Qiagen, Gilden, Germany). In agreement with the RNA concentration, the volume of each RNA sample was calculated for each RT reaction, so that all samples can start in a similar way (approximately 100 ng of total RNA) in all RT reactions. After the RT assay, samples were diluted 1/10 before they were used for qPCR.

Verification of the absence of genomic DNA in the RNA samples

To verify the removal of genomic DNA from all RNA samples we also used qPCR, studying the ACT β gene (oligonucleotides obtained from the Origene website do not amplify introns): sense primer 5' CACCATTGGCAATGAGCGGTTC3' and anti-sense primer 5' AGGTCTTTGCGGATGTCCACGT3'. Thus, the cycle's threshold (CT) in qPCR of each cDNA sample was compared with the CT in qPCR of the same amount of its corresponding RNA (Delta CT $\geq$ 5) [Laurell et al, 2012].

Selection of reference gene for qPCR normalization

We studied the GusB housekeeping gene as the reference gene for normalization. The GusB gene was used as a reliable gene for normalization in qPCR experiments [Guillot et al, 2017]. Moreover, the GusB gene has been used as a constitutive gene, having a stable expression with the HeberNasvac/CIGB2020 vaccine treatment during pharmacological studies [Fleites et al, 2021; Aguiar et al, 2022].

Quantitative polymerase chain reaction (qPCR) designed for gene expression studies

The "Qgene" software [Muller et al, 2002; Perikles, 2003] was used to process primary data from qPCR to obtain the mean normalized expression of the transcripts for each interferon gene studied.

Quantitative analysis of gene expression using qPCR

qPCR reactions were prepared using the SYBR Green from a Roche kit (Light Cycler 480 SYBR Green I Master, Germany), we also added the specific primers (synthesized at the Primers Synthesis Department of CIGB). The qPCR reaction was established in a final volume of 15 μ L as follows: 7.5 μ L of SYBR Green Master mix from the Roche kit, 1 μ L of each sense and antisense primers at the concentration of 10 pmol/ μ L, and 0.5 μ L of water from the Roche kit. Then we added 5 μ L of each cDNA sample to the qPCR reactions. All qPCR experiments were conducted in a "Rotor Gene 3000" device from Corbett Research, Australia, using a 72 well rotor. The program used for the qPCR assay was as follows: 95°C per 5', 45X (94°C per 10", 60°C per 10" and 72°C per 10").

The results of the CT's from qPCR were used to calculate the "mean normalized expression" of each interferon gene from all volunteers included in each one of the 4 groups using the "Qgene" software. Later, we compared the mean normalized expressions of each interferon gene between all 4 treated groups on day 0 before treatment, vs days 8 and 15 after the start of the treatment, in order to establish the relative expression stimulations.

Genes and Primers

Primers were designed in some cases using the Primer

3 software and in others; we used reports from relevant scientific papers [Guillot et al, 2017]. For example, the following oligonucleotides were used to amplify fragments of the human IFN α gene: (sense: 5'CCCCAGGAGGAGTTTGATGGCA3', antisense: 5'GAGGTCCTCATCCCAAGCAGCA3'), IFN β gene: (sense: 5'CTTGGATTCTACAAAGAAGCAGC3', antisense: 5'TCCTCCTTCTGGAAGTCTGCA3'), IFN γ gene: (sense: 5'TCGACCTCGAAACAGCATCT3', antisense: 5'TGTCCAACGCAAAGCAATAC3'). The human GusB gene (sense: 5'CGTGGTTGGAGAGCTCATTTGGAA3', antisense: 5'ATTCCCCAGCACTCTCGTCTGGT3') was employed as a constitutive gene for normalization. [Guillot et al, 2017].

Statistics

To normalize the distribution for the analysis of the relative expression of the interferon genes, the values of the mean normalized expression of the interferons were transformed logarithmically. After their transformation, a test of atypical values (outliers) was applied to each group on days 0 (untreated samples), and 8 and 15 days after treatment. Finally, to compare the effects of each treatment and the effects of the time of evaluation (day 0 = T0, day 8 = T1 and day 15 = T2) a two-way ANOVA was applied. The significance level used was 0.05. Calculations were performed with Minitab 17.1.0 (Trademark of Minitab Inc).

RESULTS

Safety and adverse events

According to the results of this Phase I/II clinical trial, the administration of HeberNasvac was safe and well tolerated in the elderly. The retention of patients in the trial was very high. Out of the 40 volunteers included, 39 received the complete number of doses. One volunteer, from G2 voluntarily interrupted the treatment after the third dose. There were 31 adverse events recorded in the four treatment

groups. According to their intensity, the events were mostly mild (87.09%), or moderate (12.9%). Local adverse events consisted in runny nose (0.6%), sneezing (0.3%), and otalgia (0.3%). Systemic adverse events were also reported: fever (0.2%) and asthenia (0.3%). No serious events or deaths occurred. According to their causality, the events were classified as unrelated (45.2%), probable (32.2%) and possible (22.6%). Most of the adverse events disappeared without any treatment (83.9%). At the end of the follow-up, 100% of the adverse events were completely resolved. No major differences between groups were detected.

Type I and type II interferon gene expression

The study demonstrated the capacity of HeberNasvac to increase the interferon expression stimulations in the majority of the elderly that we could evaluated in the 4 groups (Figure 1) at days 8 (T1) and 15 (T2) after treatment, when compare to day 0 (or T0).

For the IFN α and IFN β the results patterns were very similar in the 4 groups, the main difference was the intensity of the expression, higher for IFN α than for IFN β . In the case of the IFN γ , all groups at T0 started with some activation of the expression of this gene (principally in group 3 (G3)), and the subsequent evaluations at days 8 (T1) and 15 (T2), although shown an increase comparing to the T0, they do not show a great increase of the expression stimulation with values similar to the IFN β .

In general, the highest interferon expression stimulations were observed when the administration were only sublingual (G4). Nevertheless, when compare the effects of the "treatment" variable on the relative expression of each interferon gene, the two-way ANOVA showed no significant differences among the 4 treatment groups. The levels of significance were $\alpha=0.05$: IFN α ($p=0.461$), IFN β ($p=0.186$) and IFN γ ($p=0.780$), presumably because of the poor sample size per group (Figure 1).

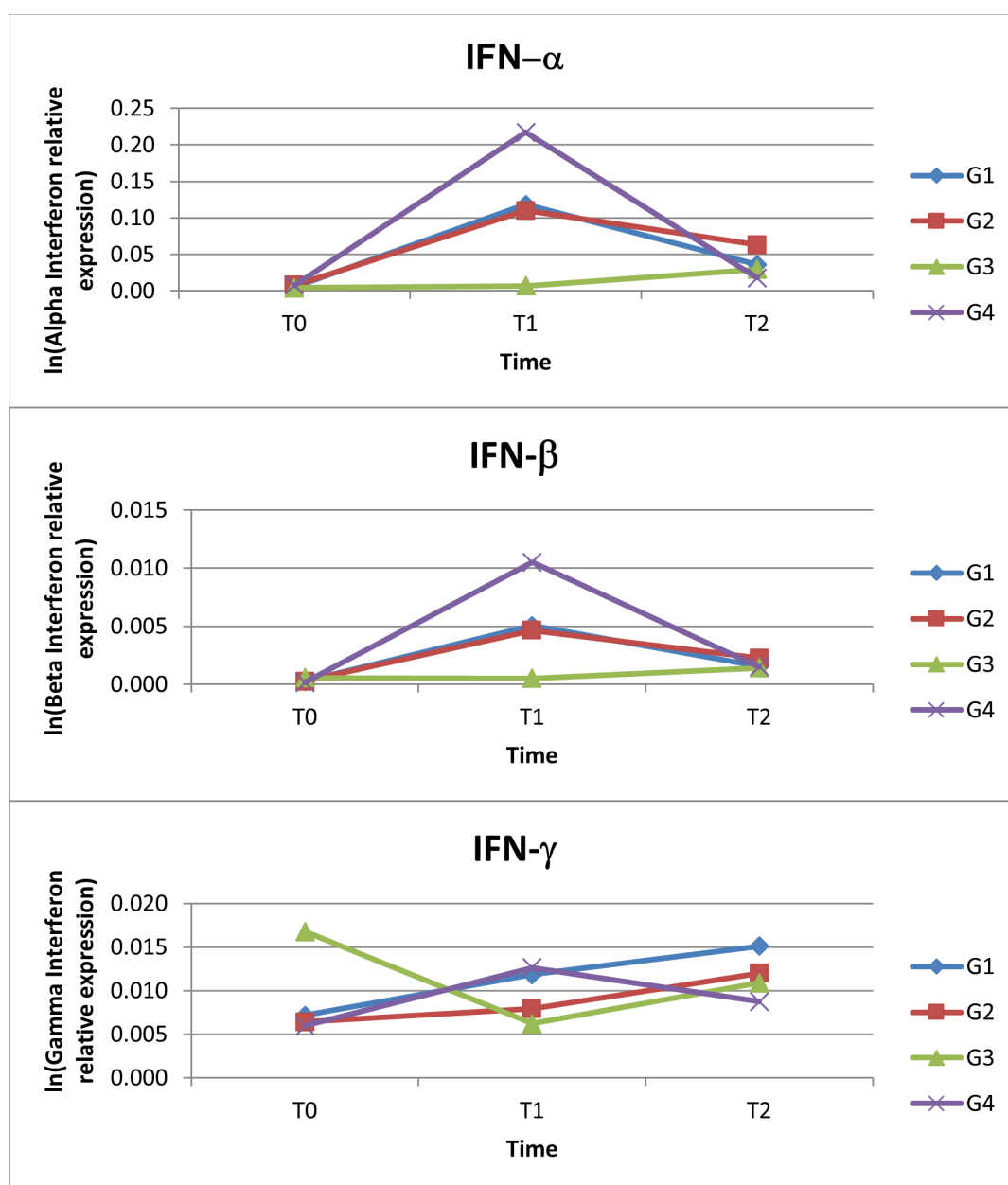


Figure 1. The graph represents the gene expression over time of type I and II interferons. To compare the effects of each treatment and the effects of the time of evaluation on days 8 and 15 a two-way ANOVA was applied. One sample (G4.12) was not included in this study because it was considered an outlier. There are no statistically significant differences when comparing the variable "treatment" between the 4 groups for the three interferons, with a level of significance $\alpha=0.05$: IFN α ($p=0.461$), IFN β ($p=0.186$) and IFN γ ($p=0.780$). Conversely, there are statistically significant differences when comparing the variable "time", for the three interferon genes studied ($\alpha=0.05$, $p=0.026$ for IFN α , $p=0.000$ for IFN β and $p=0.020$ for IFN γ). Group 1 (G1) i.e. the positive control group, was submitted to the treatment (IN spray on days 0, 7 and 14 and SL drops from day 0 to 14) [Fleites et al, 2021; Aguiar et al, 2022; Aguiar et al, 2024]. Group 2 (G2) received IN drops on days 0, 7 and 14 and SL drops from day 0 to 14. Group 3 (G3) received nasal drops alone on days 0, 7 and 14 without the SL administration and group 4 (G4) was treated only with SL drops every day from day 0 to 14. T0: (time before the treatments). T1 and T2: (8 and 15 days after the start of immunization, respectively).

On the contrary, when study the "time" variable, the two-way ANOVA showed significant differences for the three interferons studied ($\alpha=0.05$, $p=0.026$ for IFN α , $p=0.000$ for IFN β and $p=0.020$ for IFN γ) (see Figure 1). In general, these results indicate that the administration of HeberNasvac under the conditions described in the present work, induced the stronger interferon responses as of day 8 after the treatment.

DISCUSSION

In general, HeberNasvac was safe and well tolerated in the four immunization groups, regardless of the inoculation route or method. Results are consistent with the safety of the product demonstrated in previous clinical trials with HeberNasvac as an innate immunity stimulator, administered here as a positive control G1 [Fleites et al, 2021; Aguiar et al, 2022; Aguiar et al, 2024], or when HeberNasvac was used as a therapeutic vaccine against Hepatitis B [Al-Mahtab et al, 2013; Al-Mahtab et al, 2018; reviewed Aguiar et al, 2022a].

Some elderly volunteers of the study were not available for blood extraction, because they don't go to the clinic at the appointment day. For this reason, final sample size for the interferon expression experiments is relatively small, which might interfere with the reliability of the results affecting the potency of the ANOVA. As a consequence, certain effects could not be detected. However, the probability obtained for aleatory differences are still valid. So, when the two-way ANOVA detects significant differences for a significant level this must be considered a valid result, even if the group size is small. The generalized increase of gene expression of interferon genes from the PBMC of elderly observed in this study is consistent with the results of HeberNasvac in the same setting for the stimulation of innate immunity receptors in oropharyngeal swabs on days 4 and 8 after immunization [Fleites et al, 2021; Aguiar et al, 2022], or for the stimulation in blood of several interferon stimulated genes (ISGs) after 5 days of the treatment [Aguiar et al, 2024]. Stimulation of the expression of the ISGs also suppose a previous interferon gene expression stimulation [Aguiar et al, 2024]. Taking into account the results of these previous studies [Fleites et al, 2021; Aguiar et al, 2022; Aguiar et al, 2024], the stimulation of IFN expression in the present clinical trial should be analyzed before day 8. We believe that there may be peaks of IFN expressions stimulation on day 4 or 5 or later, but before day 8, as previously reported for the innate immunity receptors [Fleites et al, 2021; Aguiar et al, 2022], or for the ISGs [Aguiar et al, 2024] expression stimulation, respectively.

The present results also contribute to the optimization of HeberNasvac administration favoring the innate immune stimulation. For example, although no significant differences were observed among the 4 treatment groups in relation to the relative expression of each interferon gene, the use of the sublingual route alone (G4), at the dose and schedule used here, produced a higher stimulatory effect on day 8 after treatment compared to the day 0, when volunteers have received only seven sublingual doses. Hence, the use of the sublingual route alone may produce a similar effect in the intensity of the interferon gene expressions during the time required in the cases of post exposure prophylaxis or early therapy.

Overall, the current and previous experience with HeberNasvac, indicate two potential uses. One is the use of its immunomodulatory properties for the early therapy or post exposure prophylaxis of infections in elderly subjects [Zhao et al, 2012; Fleites et al, 2021; Aguiar et al, 2022; Aguiar et al, 2024] and patients susceptible to developing severe disease. Its other potential use is that of an agent that can induce innate immunity training relevant for oncologic diseases [van Puffelen et al, 2020; Lérias et al, 2020; Singh et al, 2022; Wang et al, 2024]; allergies [Wanka and Jappe, 2021; Martín-Cruz et al, 2023] or susceptibility to infections in order to reduce the use of antibiotics in immune-suppressed patients including those at high-risk settings, for example patients on cancer therapy [Ochoa-Grullon et al, 2021; Owen et al, 2022].

CONCLUSIONS

In conclusion, these results confirm the capacity of HeberNasvac VLPs with immunostimulatory properties, to induce a generalized stimulation of interferon responses at the systemic compartment of elderly volunteers after sublingual and intranasal administration by separate, or combination of both routes, on day 8 after treatment. Additional clinical and formulation studies are required with increasing of the sample size to further develop a rational, cost-effective and industrially acceptable product, with a broad-spectrum antiviral effect, based on the stimulation of innate immunity with pure and safe formulations of VLPs. Such a product would be useful in the setting of post exposure prophylaxis or early therapy of acute respiratory or febrile infectious diseases as well as with the scope of training innate immunity responses for the control of chronic diseases.

All authors attest they meet the ICMJE criteria for authorship.

Author Contributions

Conceptualization: Ideas; formulation or evolution of overarching research goals and aims: Jorge Agustín Aguiar Santiago, Julio Cesar Aguilar Rubido, Zurina Cinza Estevez, Gerardo E Guillén Nieto, Eduardo Pentón Arias. Data curation: Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later re-use: Jorge Agustín Aguiar Santiago, Julio Cesar Aguilar Rubido, Alina Díaz Machado, Carlos Alberto González Delgado, Sonia Pérez Rodríguez, Iris Valdés Prado, Gerardo García Ilera, Iván Luis Santos Martínez, Chabeli Rodríguez Ibarra, Maria Acelia Marrero Miragaya, Zurina Cinza Estevez, Gerardo E Guillén Nieto, Eduardo Pentón Arias. Formal analysis: Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data: Jorge Agustín Aguiar Santiago,

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Conflicts of Interest

The authors declare no conflicts of interest.

REFERENCES

- Aguilar J, Marrero MA, Figueroa DA, et al. Preparing for the next pandemic: Increased expression of interferon stimulated genes after local administration of Nasalferon or HeberNasvac. *DNA and Cell Biology* 2024; 43 (2): 95 - 103. DOI: 10.1089/dna.2023.0283
- Aguilar J, Silva J, Gell O, et al. An in-house qPCR System to Evaluate Transcripts Stimulated by an Immune Innate Agonist (CIGB2020) during the Covid19 Pandemic Scenario. *Appl Cell Biol*. 2022, 10 (2): 45 - 50. DOI:10.53043/2320-1991.acb90024
- Aguilar JC a, Aguiar J, Akbar SMF. Action Mechanisms and Scientific Rationale of Using Nasal Vaccine (HeberNasvac) for the Treatment of Chronic Hepatitis B. *Vaccines* 2022; 10 (12): 2087. DOI.org/10.3390/vaccines10122087
- Aguilar JC b, Pentón E, Akbar SMF. Innate Immune Stimulation Should not be Overlooked in Postexposure Prophylaxis and Early Therapy for Coronavirus Infections. *MEDICC Review* 2022; 24: 70 - 75. DOI: 10.37757/MR2022.V24.N1.5

Al-Mahtab M, Akbar SMF, Aguilar JC, et al. Therapeutic potential of a combined hepatitis B virus surface and core antigen vaccine in patients with chronic hepatitis B. *Hepatology International* 2013; 7: 981 - 989. DOI: 10.1007/s12072-013-9486-4

Al Mahtab M, Akbar SMF, Aguilar JC, et al. Treatment of chronic hepatitis B naïve patients with a therapeutic vaccine containing HBs and HBc antigens (a randomized, open and treatment-controlled phase III clinical trial). *PLoS One* 2018; 13 (8): e0201236. DOI: 10.1371/journal.pone.0201236

Fleites Y, Aguiar J, Cinza Z, et al. HeberNasvac, A Therapeutic Vaccine for Chronic Hepatitis B, stimulates Local and Systemic Markers of Innate Immunity: Potential Use in SARS-CoV-2 Postexposure Prophylaxis. *Euroasian J Hepato-Gastroenterol* 2021; 11(2): 59 - 70. DOI: 10.5005/jp-journals-10018-1344

Guillot C, Martel N, Berby F, et al. Inhibition of hepatitis B viral entry by nucleic acid polymers in HepaRG cells and primary human hepatocytes. *PLoS ONE* 2017; 12 (6): e0179697. DOI: 10.1371/journal.pone.0179697

Hackett CJ. Innate immune activation as a broad-spectrum biodefense strategy: Prospects and research challenges. *J Allergy Clin Immunol* 2003; 112 (4): 686 - 694. DOI: 10.1016/S0091-6749(03)02025-6

Kikkert M. Innate immune evasion by human respiratory RNA viruses. *Journal of Innate Immunity* 2020; 12 (1): 4-20. DOI: 10.1159/000503030

Kumaki Y, Salazar AM, Wandersee MK, et al. Prophylactic and therapeutic intranasal administration with an immunomodulator, Hiltonol® (Poly IC: LC), in a lethal SARS-CoV-infected BALB/c mouse model. *Antiviral Research* 2017; 139: 1 - 12. DOI: 10.1016/j.antiviral.2016.12.007

Laurell H, Lacovoni, J, Abot A, et al. Correction of RT-qPCR data for genomic DNA-derived signals with ValidPrime. *Nucleic Acid Research* 2012; 40 (7): 1 - 10. DOI: 10.1093/nar/gkr125915

Luo W, Liu Q, Zhou Y, et al. Spatiotemporal variations of “triple-demic” outbreaks of respiratory infections in the United States in the post-COVID-19 era. *BMC Public Health*; 2023 (23): 2452. <https://DOI.org/10.1186/s12889-023-17406-9>

Lérias JR, De Sousa E, Paraschoudi G, et al. Trained immunity for personalized cancer immunotherapy: current knowledge and future opportunities. *Frontiers in Microbiology* 2020; 10: 2924. DOI: 10.3389/fmicb.2019.02924

Martín-Cruz L, Sevilla-Ortega C, Angelina A, et al. From trained immunity in allergy to trained immunity-based allergen vaccines. *Clinical & Experimental Allergy* 2023; 53 (2): 145 - 155. DOI: 10.1111/cea.14261

Marx S, Kümmerer BM, Grützner C, et al. RIG-I-induced innate antiviral immunity protects mice from lethal SARS-CoV-2 infection. *Molecular Therapy-Nucleic Acids* 2022; 27: 1225 - 1234. DOI: 10.1016/j.omtn.2022.02.008

Muller P, Janovjak H, Miserez A, et al. Processing of Gene Expression Data Generated by Quantitative Real-Time RT-PCR. *BioComputing/ Bioinformatics Short Technical Report. BioTechniques* 2002; 32 (6): 1372 - 1379. PMID: 12074169

Nelemans T, Kikkert, M. Viral innate immune evasion and the pathogenesis of emerging RNA virus infections. *Viruses* 2019; 11 (10): 961. DOI: 10.3390/v11100961

Ochoa-Grullon J, Benavente C, Gonzalez A, et al. Trained immunity-based vaccine in B cell hematological malignancies with recurrent infections: a new therapeutic approach. *Frontiers in Immunology* 2021; 11: 611566. DOI: 10.3389/fimmu.2020.611566

Ouyang Y, Liao H, Hu Y, et al. Innate immune evasion by human respiratory syncytial virus. *Frontiers in Microbiology* 2022; 13: 865592. <https://DOI.org/10.3389/fmicb.2022.865592>

Owen AM, Luan L, Burelbach KR, et al. MyD88-dependent signaling drives toll-like receptor-induced trained immunity in macrophages. *Frontiers in Immunology* 2022; 13: 1044662. DOI: 10.3389/fimmu.2022.1044662

Perikles S. Q-Gene: processing quantitative real-time RT-PCR data. *BIOINFORMATICS APPLICATIONS NOTE* 2003; 19 (11): 1439 - 1440. DOI: 10.1093/bioinformatics/btg157

Sheahan T, Morrison TE, Funkhouser W, et al. MyD88 is required for protection from lethal infection with a mouse-adapted SARS-CoV. *PLoS Pathogens* 2008; 4 (12): e1000240. DOI: 10.1371/journal.ppat.1000240

Singh AK, Praharaj M, Lombardo KA, et al. Re-engineered BCG overexpressing cyclic di-AMP augments trained immunity and exhibits improved efficacy against bladder cancer. *Nature Communications* 2022; 13 (1): 878. DOI: 10.1038/s41467-022-28509-z

Tamir H, Melamed S, Erez N, et al. Induction of Innate Immune Response by TLR3 Agonist Protects Mice against SARS-CoV-2 Infection. *Viruses* 2022; 14: 189. DOI: 10.3390/v14020189

Thorne LG, Bouhaddou M, Reuschl AK, et al. Evolution of enhanced innate immune evasion by SARS-CoV-2. *Nature* 2022; 602: 487 - 495. DOI: 10.1038/s41586-021-04352-y

van Puffelen JH, Keating ST, Oosterwijk E, et al. Trained immunity as a molecular mechanism for BCG immunotherapy in bladder cancer. *Nature Reviews Urology* 2020; 17 (9): 513 - 525. DOI: 10.1038/s41585-020-0346-4

Wang T, Wang Y, Zhang J, et al. Role of trained innate immunity against mucosal cancer. *Current Opinion in Virology* 2024; 64: 101387. DOI: 10.1016/j.coviro.2024.101387

Wanka L, Jappe U. Trained immunity and allergy: State of the art and future perspectives. *Allergy* 2021, 76 (4): 1265 - 1267. DOI: 10.1111/all.14617

Zhang S, Yang Z, Li ZN, et al. Are Older People Really More Susceptible to SARSCoV-2? Aging and Disease 2022; 13 (5): 1336 - 1347. DOI: 10.14336/AD.2022.0130

Zhao J, Wohlford-Lenane C, Zhao J, et al. Intranasal treatment with poly (I·C) protects aged mice from lethal respiratory virus infections. *Journal of Virology* 2012; 86 (21): 11416 - 11424. DOI: 10.1128/JVI.01410-12