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Preventive and Therapeutic Vaccination for COVID-19 Using Novel Antigen, Strong but Amicable Adjuvant and Exploring Alternate Route of Administration

By

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ABSTRACT: The World Health Organization states that about 773 million people were infected by SARS-CoV-2 and the death toll touched about 7.4 million, although both the incidence and mortality crossed WHO observation. Several vaccines have been developed to counter SARS-CoV-2 and medicines of multiple disciplines have been used in COVID-19 patients. It is not unlikely another virus like SARS-CoV-2 may induce another pandemic the globe. In this pretext, we initiated some studies to develop a new and novel therapeutic that is endowed with both preventive and curative purposes. A new area of novel drug development against COVID-19 was formulated by discovering a novel agent that induce both innate and adaptive immunity and act beyond virus specificity. During the COVID era, we checked the preventive and therapeutic nature of a vaccine containing an antigen of hepatitis B virus (hepatitis B surface antigen, HBsAg) with a strong adjuvant (hepatitis B core antigen, HBcAg) (both GMP level and usable in human) by administering via nasal route (preferential mucosal route). Clinical trial in 2000 and 2022 cited its potential preventive and therapeutic activities against SARS-CoV-2 and COVID-19, especially in downregulating progression of diseases. The studies of mechanisms indicated its capacity of inducing and regulating proinflammatory and anti-inflammatory cytokines when administered via nose. We have further worked on more potent antigens, adjuvants and easy-to-use devices. The present communication would unmask the mechanism of action of this multipurpose vaccine with comparative assessment of utility of other preventive and therapeutic modalities in COVID-19 patients to optimize drug development machineries against this pathology of pandemic of viruses that enter via nose or other mucosal areas. This will ultimately indicate that a preventive and therapeutic intervention may be possible even when antigens of the responsible virus is elusive and thus a procedure would be realized to fight epidemics and pandemics during their initial stages.

KEYWORDS: COVID-19, Pre-and Post-Exposure Prophylaxis, Therapeutic Vaccine.

INTRODUCTION

Coronavirus 2019 (COVID-19) is a disease that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2). SARS-Cov-2 is novel, positive-sense, single stranded RNA beta-coronavirus, which was first identified in humans in December of 2019 and in the early part of the

present decade, caused the latest pandemic faced by the human race. It now appears that the human race will have to live with this virus for several decades, if not longer [1, 2].

COVID-19 has wide spectrum of presentations. On one extreme, there are many patients who remain asymptomatic, while on the other extreme some experience severe form of the disease with poor prognosis. However, vast majority of COVID-19 patients develop mild to moderate symptoms [3, 4, 5]. Host immunity is crucial for SARS-Cov-2 infection and for the severity of COVID-19. This is the reason why elderly, obese and those who have comorbidities experienced most of the fatalities form COVID-19 [6, 7, 8].

SARS-Cov-2 and the role of innate Immunity

The first line of defense of humans against any given viral infection is innate immune response. There are pattern-recognition receptors (PRR) on plasma membranes, endosomal membranes and cytosol which first recognize viral components or replication intermediates known as pathogen-associated molecular patterns (PAMP) and then activate innate immunity. The initial step of viral infection depends on the complex interplay between viruses, viral receptors, PRRs and PAMPs. Natural killer (NK) cells, NK T-cells, neutrophils, dendritic cells (DC) and macrophages prevent viral attachment to specific host receptors and inhibit viral replication by obstructing viral localization or by destroying viruses. After entry of SARS-Cov-2 into the nasal mucosa, innate, regulatory and adaptive immunities interplay in COVID-19 pathogenesis. So, one of the approaches to counter SARS-Cov-2 infection may be to block the localization of this virus in the nasal cavity [9, 10].

NASVAC in chronic hepatitis B

Hepatitis B virus (HBV) is a non-cytopathic hepatitis virus, which is the leading cause of all forms of chronic liver diseases globally, more so in the Asia-Pacific. Liver damage in HBV infected individuals is immune mediated. This is the reason why out of the 2 billion people infected with HBV across the globe, 34-70 million *i.e.* 12-25% show evidence of liver damage, while the rest don't [11]. There has been significant efforts to use immune therapy as an innovative treatment for CHB. Cytokines, growth factors, immune-modulators and other immunogenic agents, including IL-2, granulocyte colony stimulating factor (GCSF) have all been tried in vein [12, 13, 14, 15, 16, 17, 18, 19, 20, 21]. Clinical trials with these immune therapies were mostly conducted as pilot studies lacking published long-term follow up data.

The only exception in this regard to date is NASVAC. It is a therapeutic vaccine developed by Centre for Genetic Engineering and Biotechnology (CIGB), Havana, Cuba comprising of HBV 's' (HBsAg) and 'c' (HBcAg) antigens produced by recombinant DNA technology. NASVAC is the outcome of years of research involving animals and humans using these two HBV antigens *i.e.* HBsAg and HBcAg to induce innate immunity, translation of innate immunity to adaptive immunity and activation of regulatory immunity through antigen-presenting DCs. In HBV-transgenic mice (HBV-TM) model, NASVAC had potent antiviral effect against HBV, without causing any liver damage [22]. Subsequently the safety of NASVAC was also established in a phase I human trial [23, 24]. NASVAC was found to safe and effective in treatment-naïve chronic HBV infected (CHB) patients in two phase I/II and phase III clinical trials [23, 25, 26]. More recently a phase IV clinical trial has established that NASVAC is also effective in achieving functional cure in 16.88% CHB patients [27].

In CHB patients, the host immune system is dysfunctional. There is overexpression of programmed death receptor 1 (PD-1) and other inhibitory receptors on T cells, which renders the immune response ineffective [28]. The intra-nasal (IN) route is very effective in inducing CD4+ T cells. In HBV-TM, an increase in the number of HBs-specific CD4+ T cells have been found in the liver following IN administration of NASVAC. However, this was not the case when NASVAC was administered through the sub-cutaneous (SC) route. The HBs-specific CD4+ T cells secrete interferon (IFN)-ϒ, interleukin (IL)-2 and tumor necrosis factor (TNF)-α. Besides, there was also increase in CD49a expressing CD4+ T cells in HBV-TM liver following administration of NASVAC through IN route. This is further evidence that nasal cavity is the site for induction and facilitation of hepatotropic cellular homing [29 C41].

Besides, CHB patients also demonstrated significant drop in HBV DNA from baseline following IN-administration of NASVAC compared to patients who received SC-administered NASVAC. Besides HBeAg seroconversion was also achieved only in IN-administered NASVAC patients, but not in those receiving NASVAC through SC route. These findings establish superiority IN route over SC route for induction of therapeutic immune response in the liver [30, 31, 32, 33, 34, 35, 36].

As of date, there is no specific medication indicated for COVID-19. Most of the drugs that have been used to treat SARS-Cov-2 infected individuals were repurposed antivirals used with emergency approval [37, 38, 39]. Besides since the onset of COVID-19 pandemic, a wide range of other drugs have also been repurposed for it's treatment. These include hydroxychloroquine originally developed for malaria, ivermectin which is an anti-parasitic agent, remdesivir used against hepatitis c virus (HCV) and respiratory syncytial virus, favipiravir which is an antiviral for Ebola and Nipah viruses and a non-specific antiviral agent IFN to name a few. However, unfortunately none of these could stand the test of time.

Based on the studies and experience with NASVAC over the years, it was hypothesized that IN administration of this therapeutic vaccine may be effective in inducing innate immunity to block SARS-Cov-2.

Newer indication for the novel one

NASVAC is a novel therapeutic vaccine, which is a rather new entry to the slow but steadily growing list of antivirals for HBV. It is currently in clinical use in some countries including Cuba [40]. During the height of the COVID-19 pandemic, a clinical trial of NASVAC was conducted in 2020 for over 6 months in Bangladesh. In this clinical trial, 20 volunteers received total 17 administrations of NASVAC, 3 IN on 0,7 and 14 days and 14 sub-lingual (SL) daily for 14 days. All the volunteers were at high risk of getting infected with SARS-Cov-2 due professional and/or household contacts. They were allowed to lead normal life with normal interaction with COVID-19 patients. NASVAC was found to be very safe. None of the study participants developed any severe adverse event (AE). Local AE *i.e.* nasal drops and sneezing were experienced in 2.14% and 0.53% participants respectively, which were mild in severity disappearing within minutes of onset without any active intervention.

Of the 20 volunteers, 4 tested positive for SARS-Cov-2 during the observation period of 6 months. All 4 of them had co-morbidities. Of them, 1 patient got admitted into hospital and required 2 liters oxygen and symptomatic treatment. He was discharged after 5 days [23].

Assessment of immune-modulatory effect of NASVAC

Ten volunteers received a single IN administration of NASVAC. Peripheral blood mononuclear cells (PBMC) were collected from all of them, the before and the day following NASVAC administration. Immune-modulatory effect of NASVAC was assessed in this study by measuring cytokine levels in the collected PBMC. The study revealed increase in IFN- ϒ, TNF- α, transforming growth factor-β (TGF- β), IL-2, IL-4 and IL-10 levels following administration of NASVAC [23]. This finding confirmed the immune-modulatory role of NASVAC, which is consistent with previous experience of NASVAC in CHB patients, in whom NASVAC normalized hepatitis significantly [25, 26]. It is believed that in COVID-19, peripheral innate immune cells suffer from functional impairment. NASVAC induced rise in IFN- ϒ and other cytokines further justifies possible role of NASVAC in preventing SARS-Cov-2 infection pre-and/or post-exposure and in arresting progression of COVID-19.

Digging deep into innate Immunity

Subsequently another phase I/II randomized, open-label clinical trial was conducted in Cuba. In all 46 hospitalized COVID-19 patients above the age of 60 years were included in this study. Of them, 24 patients received NASVAC following the same schedule as in the Bangladesh trial, while the rest 22 were controls. This was post-exposure prophylaxis clinical trial to assess the local and systemic immunomodulatory effects of NASVAC in COVID-19. The safety of NASVAC was once again established in this study. AEs included nasal drops (0.58%), sneezing (0.3%), otalgia (0.3%), fever (0.3%) and asthenia (0.3%), none being serious and relieved either spontaneously (62.5%) or with pharmacological intervention (32.5%) [41].

The study revealed that significantly higher proportion of NASVAC-treated patients had TLR3, TLR4 and TLR5 gene expression, while housekeeping gene GusB remained stable. In the control group, higher proportion of patients had undetectable signals for any of the TLRs. HLA-DR expression on monocytes and lymphocytes was also observed in NASVAC-treated patients [41].

NASVAC in COVID-19

The Bangladesh clinical trial was a small proof of concept study, which showed that IN administration of the repurposed therapeutic vaccine NASVAC, induced innate immune response in the nasal and oropharyngeal regions preventing localization and replication of SARS-Cov-2 in the very initial stage. It also reduced the severity of COVID-19 in susceptible individuals with co-morbidities. It showed the potential of NASVAC in post-exposure prophylaxis against SARS-Cov-2 and as a preemptive therapeutic option for COVID-19.

Studies have shown survival benefits from SARS-Cov-2 and influenza A virus infections in murine models following stimulation of the agonists of local innate immunity receptors *i.e.* TLRs involved in detecting such RNA viruses *e.g.* TLR3 and TLR7/8 [42, 43]. HBcAg can stimulate TLR2, TLR3 and TLR7, activate MyD88-dependent and MyD88-independent pathways and increase HLA and IFN expression [44, 45, 46].

Stimulation of HepaRG cell line with HBcAg *in vitro* is associated with multi-TLR agonist effect [47, 48, 49]. Increase in innate immune receptors, namely RNA-sensing TLR3, TLR7 and TLR8 on tonsils of the patients in the Cuban study following NASVAC administration is consistent with this finding. Besides, dramatic survival benefits have been seen in lethal SARS infected mice model following administration of TLR3, TLR7/8 and TLR9 agonists [50, 51]. Transcriptomic profiling studies involving SARS-Cov-2 infected cells *in vitro* also support the role of TLR3 and TLR7/8 agonists for early immune stimulation [52]. Furthermore, TLR3 gene downregulation is more pronounced in bovine coronavirus compared to bovine rotavirus [53]. This suggests that TLR3 is the target in coronavirus infection.

It is well established that COVID-19-related mortality was higher in the elderly. Old age-related alterations in immune response after stimulation of pathogen recognition receptors may explain this phenomenon. It was observed that NASVAC had protective effect in the elderly. Increase in HLA class II expression in monocytes and lymphocytes of NASVAC-treated elderly volunteers may have contributed to this, as in severe COVID-19 there is reduced expression of HLA-DR on monocytes and myeloid DC (mDC) [54]. This is consistent with previous observations that NASVAC stimulates innate and adoptive immunity both *in vivo* and *in vitro* [55, 56]. Besides, it has also been observed that NASVAC stimulates TLR, HLA class I/II and costimulatory molecule gene expression *in vitro* in HepaRG model [47].

Studies have revealed that in COVID-19, peripheral innate immune cells have functional impairment. It is also known that TLR3, TLR7 and TLR8 signaling pathways induce IFN production. During the COVID-19 pandemic, clinical trials yielded beneficial results with IFN in SARS-Cov-2 infection [57]. It is likely that NAVAC may help COVID-19 patients by inducing IFN production and also by improving detection of viral RNA by the innate immunity [58, 47, 59, 60]. It may be noted that increased expression of TLR3, TLR7 and TLR8 genes in nasopharyngeal tonsils are surrogate markers of protection against SARS-Cov-2 in lethal infection mice model [41].

It may further be noted that synergistic stimulation of MyD88-dependent and -independent pathways via TLR3, TLR7 and TLR8 decreases viremia and induces clinical improvement in Dengue. This may also be useful in hepatocellular carcinoma (HCC), where TLR3 expression correlates with apoptosis, proliferation, angiogenesis and prognosis [61, 62, 63].

Conclusion

Safety of NASVAC in COVID-19 has been well established. Besides it may also be inferred that NASVAC will be effective both in pre-/post-exposure prophylaxis against SARS-Cov-2 and also in preventing the progression of severe COVID-19. Not only that, NASVAC may also eventually become an effective immunomodulatory prophylactic intervention against other viruses like Dengue as well as for treatment of malignancies like HCC.

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