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Pre-clinical development of a hydrogen peroxide-inactivated West Nile virus vaccine

Elizabeth A. Poore [a,1], Dawn K. Slifka [a,1], Hans-Peter Raué [b], Archana Thomas [b], Erika Hammarlund [b], Benjamin K. Quintel [a], Lindsay L. Torrey [a], Ariel M. Slifka [a], Justin M. Richner [d], Melissa E. Dubois [a], Lawrence P. Johnson [c], Michael S. Diamond [d,e,f,g], Mark K. Slifka [a,b], Ian J. Amanna [a,*]

[a] Najít Technologies, Inc, Beaverton, OR, USA
[b] Division of Neuroscience, Oregon National Primate Research Center, Department of Molecular Microbiology and Immunology, Oregon Health & Science University, Beaverton, OR, USA
[c] BioFinity Consulting, Beaverton, OR, USA
[d] Departments of Medicine, Washington University School of Medicine, St. Louis, MO, USA
[e] Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA
[f] Pathology & Immunology, Washington University School of Medicine, St. Louis, MO, USA
[g] The Center for Human Immunology and Immunotherapy Programs, Washington University School of Medicine, St. Louis, MO, USA

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ABSTRACT

West Nile virus (WNV) is a mosquito-transmitted pathogen with a wide geographical range that can lead to long-term disability and death in some cases. Despite the public health risk posed by WNV, including an estimated 3 million infections in the United States alone, no vaccine is available for use in humans. Here, we present a scaled manufacturing approach for production of a hydrogen peroxide-inactivated whole virion WNV vaccine, termed HydroVax-001 WNV. Vaccination resulted in robust virus-specific neutralizing antibody responses and protection against WNV-associated mortality in mice or viremia in rhesus macaques (RM). A GLP-compliant toxicology study performed in rats demonstrated an excellent safety profile with clinical findings limited to minor and transient irritation at the injection site. An *in vitro* relative potency (IVRP) assay was developed and shown to correlate with *in vivo* responses following forced degradation studies. Long-term *in vivo* potency comparisons between the intended storage condition (2–8 °C) and a thermally stressed condition (40 ± 2 °C) demonstrated no loss in vaccine efficacy or protective immunity over a 6-month span of time. Together, the positive pre-clinical findings regarding immunogenicity, safety, and stability indicate that HydroVax-001 WNV is a promising vaccine candidate.

1. Introduction

West Nile virus (WNV) is a member of the genus *Flavivirus* (family *Flaviviridae*), a group that includes many clinically relevant arthropod-borne viruses such as yellow fever (YFV), dengue (DENV), Japanese encephalitis (JEV), tick-borne encephalitis, and Zika viruses [1]. All members are single-stranded positive-sense RNA viruses with genomes of approximately 11 kilobases that form enveloped mature infectious particles roughly 50 nm in diameter. The genome is translated into a large polyprotein that is subsequently processed by both cellular and viral proteases into 3 structural proteins (capsid, premembrane, and envelope [Env]) and 7 nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5). WNV is now endemic throughout the continental United

Abbreviations: BDS, bulk drug substance; BPL, β-propiolactone; cGMP, current good manufacturing practices; DENV, dengue virus; DP, drug product; Env, envelope protein; FFA, focus forming assay; GLP, good laboratory practices; H_2O_2, hydrogen peroxide; IVRP, *in vitro* relative potency; JEV, Japanese encephalitis virus; LOD, limit of detection; MAb, monoclonal antibody; MCB, master cell bank; MOI, multiplicity of infection; MVB, master virus bank; NT_{50}, 50% neutralizing titer; PFU, plaque forming unit; PPV, porcine parvovirus; pre-MVB, pre-master virus bank; s.c., subcutaneous; T1/2, half-life; TPP, triple plaque purification; WCB, working cell bank; WNV, West Nile virus; WNV-KV, WNV Kunjin strain; WVB, working virus bank; YFV, yellow fever virus.

* Corresponding author at: Najít Technologies, Inc., 505 NW 185th Avenue, Beaverton, OR 97006, USA.
E-mail address: iamanna@najittech.com (I.J. Amanna).
[1] These authors contributed equally to this work.

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States, with periodic peaks and troughs in disease incidence [2]. Although ~75% of infections are believed to be asymptomatic [3], symptomatic infection can be severe with a mortality rate among reported WNV cases of approximately 4% [4], increasing to as high as 23% among elderly patients ⩾70 years of age [5].

Currently, no vaccine is available to prevent WNV disease in humans. Although a correlate of WNV immunity has not been established formally, it is recognized that neutralizing antibodies have an important role in protective immunity [6–12]. We previously have shown that neutralizing antibodies are sufficient for protection against WNV infection in mice [6,8,13] and have reported on the development of a hydrogen peroxide (H_2O_2) inactivated WNV vaccine candidate that demonstrated robust neutralizing antibody responses in mice, with protection against stringent intracranial challenge in both young and aged animals [14]. Herein, we describe the pre-clinical development and testing of this vaccine candidate and provide supportive evidence and proof-of-principle for the use of H_2O_2-based virus inactivation as an effective approach to develop a new WNV vaccine for humans.

2. Materials and methods

2.1. Vero cell stocks and banks

WHO Vero Seed Lot 10-87 was selected as the parental cell line for vaccine production on the basis of the extensive historical testing and characterization associated with this line [15]. Serum-free cell bank development was carried out under contract by SAFC Pharma (Carlsbad, CA) using optimized procedures established by NTI, with both a master cell bank (MCB) and working cell bank (WCB) manufactured in compliance with current good manufacturing practices (cGMP). The MCB was tested and confirmed to be negative for adventitious agents including porcine circovirus types 1 and 2.

2.2. Virus stocks and banks

The chemically resistant model virus, porcine parvovirus (PPV, strain NADL-2) was obtained from the American Tissue Culture Collection (Manassas, VA) and titered on swine testicle cells. WNV strains Texas TX02, Egypt101, New York 1999 (NY99), Madagascar AnMg798 and South Africa H442 SA58, have been described previously [13,16,17]. A low-passage, attenuated, lineage 1 Kunjin virus isolate (WNV-KV, strain CH16532 [14]) was selected for vaccine development. The WNV-KV master seed was sequentially triple-plaque-purified on serum-free adapted Vero cells and used to develop a pre-master virus bank (pre-MVB). The WNV-KV pre-MVB, propagated on the Vero WCB, was used to prepare the WNV-KV master virus bank (MVB), which was in turn used to manufacture a working virus bank (WVB). SAFC Pharma performed all manufacturing steps under cGMP compliance. Both virus banks were sequenced according to good laboratory practices (GLP, Genewiz, Inc.) and confirmed to be free of potential adventitious agents.

2.3. Inactivation kinetics and tests for residual live virus

Inactivation kinetics of WNV-KV by H_2O_2 were studied under small-scale conditions using our previously described hydrogen peroxide-based approach with catalase treatment to remove residual H_2O_2 prior to performing live virus quantitation [14]. To confirm complete inactivation for manufacturing purposes, 5% of the total bulk drug substance (BDS) was tested using a validated residual live virus assay developed in conjunction with WuXi AppTec, similar to the approach recently described for an inactivated YFV vaccine candidate [18].

2.4. Vaccine

HydroVax-001 WNV is a whole virion vaccine inactivated with H_2O_2 and adsorbed to aluminum hydroxide adjuvant. Two sequential manufacturing lots (Lot 159-13001 and Lot 159-13005) were produced by SAFC Pharma. Lot 159-13001 was manufactured in cGMP facilities using draft batch records to confirm the manufacturing process at scale, with Lot 159-13005 produced under cGMP compliance for subsequent clinical use. For each production, aliquots of the WCB were thawed, expanded in serum-free medium, and passaged into a GE WAVE bioreactor using microcarrier beads. Cells were infected with the WNV-KV WVB, with virus harvests collected at the anticipated peak of viral replication using a batch-refeed method. Harvests were clarified, pooled, and treated with Benzonase to minimize host-cell DNA contamination. The Benzonase-treated, clarified pooled harvest was then concentrated and buffer-exchanged using tangential flow filtration. Just prior to initiating virus inactivation, material was filtered and H_2O_2 was added at a final concentration of 3.0%. Inactivation proceeded with gentle agitation at a controlled temperature (25 ± 2 °C) for 7 h. Halfway through inactivation, an additional filtration was performed to limit potential aggregates. Directly following inactivation, viral material was purified using ion-exchange chromatography with a subsequent tangential flow filtration diafiltration step for formulation of BDS into a proprietary stabilization buffer, followed by final sterile filtration. BDS was then formulated with 0.1% aluminum hydroxide (Alhydrogel®, Brenntag Biosector, Denmark) to produce the final drug product (DP) and filled into stoppered glass vials. The BDS and DP conformed to specifications on appearance, osmolality, pH, protein concentration, residual Benzonase, residual host cell DNA, residual protein, residual growth medium components, potency, residual live virus, identity (SDS-PAGE and confirmatory Western blot using the WNV-specific MAb, 7g11 [14]), endotoxin, general safety and sterility.

To assess vaccine potency a WNV-specific *in vitro* relative potency (IVRP) assay was developed incorporating both the measurement of total protein and WNV-specific antigen via ELISA, similar to other non-replicating vaccines [18,19], with direct testing of the BDS or desorbed DP. The WNV Env-specific, neutralizing mouse monoclonal antibody (MAb) 7g11 [14] was used as both the capture and detection reagent. Log-log transformed ELISA optical density (OD) and protein concentration were plotted in the linear portion of the curve. The titer was defined as the protein concentration needed to reach an OD of 0.50 and normalized to a reference standard established using an engineering lot of BDS (similar to [19]). Samples from clinical-grade vaccine lots were tested in triplicate by WuXi AppTec using GLP-compliant practices.

2.5. ELISA and neutralization assays

Epitope mapping of WNV-KV after different modes of inactivation was performed with previously described WNV Env-specific monoclonal antibodies (MAbs) [8,10], following a 1:250 dilution of the target antigen to reduce residual inactivation components. Immunity following vaccination was assessed by ELISA using WNV-KV lysate similar to previous studies [13]. Serum neutralization titers (NT_{50}) for WNV-NY99 were determined using a plaque reduction assay as described previously [13], whereas neutralization titers for all other WNV strains were performed using a focus-forming assay (FFA) [14]. WNV-KV neutralization titers were initially tested and found comparable using either the plaque assay or FFA (Supplemental Fig. 1), with all subsequent testing performed using FFA.

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