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A plant-derived quadrivalent virus like particle influenza vaccine induces cross-reactive antibody and T cell response in healthy adults

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ABSTRACT

Recent issues regarding efficacy of influenza vaccines have re-emphasized the need of new approaches to face this major public health issue. In a phase 1–2 clinical trial, healthy adults received one intramuscular dose of a seasonal influenza plant-based quadrivalent virus-like particle (QVLP) vaccine or placebo. The hemagglutination inhibition (HI) titers met all the European licensure criteria for the type A influenza strains at the 3 μg/strain dose and for all four strains at the higher dosages 21 days after immunization. High HI titers were maintained for most of the strains 6 months after vaccination. QVLP vaccine induced a substantial and sustained increase of hemagglutinin-specific polyfunctional CD4 T cells, mainly transitional memory and T_{EMRA} effector IFN-γ^+ CD4 T cells. A T cells cross-reactive response was also observed against A/Hong-Kong/1/1968 H3N2 and B/Massachusetts/2/2012. Plant-based QVLP offers an attractive alternative manufacturing method for producing effective and HA-strain matching seasonal influenza vaccines.

1. Introduction

Influenza A viruses are a major public health threat and, in the USA alone, seasonal epidemics account for >200.000 hospitalizations and >30.000 deaths annually [1]. Influenza B viruses can also cause seasonal epidemics in adults every two to four years. Based on data across four seasons, the clinical symptoms and hospital admission rates are similar in subjects infected with either influenza A or B [2]. Vaccination remains the most cost-effective means to control the health burden attributable to influenza. Seasonal influenza vaccines have historically included three strains: two influenza A strains and only one influenza B strain. As two antigenically-distinct influenza B lineages (B/Victoria and B/Yamagata) have co-circulated in North America since 2000, there have been frequent mismatches leading to a reduced vaccine effectiveness [3,4]. Indeed, split-virion vaccination against one B lineage provides little-to-no cross-protection against the alternate lineage [5,6]. It has been estimated that inclusion of both Victoria and Yamagata lineages in a quadrivalent inactivated vaccine (QIV) between 1999 and 2009 could have resulted in a major and significant reduction of the public health burden of influenza infections [7]. For the 2012–2013 Northern hemisphere influenza season, the World Health Organization (WHO) recommended the inclusion of a B strain from each lineage in the seasonal vaccine for the first time. Since that time, several different QIV formulations have been shown to be immunogenic for all four vaccine strains in children as young as 6 months and in adults [8–11]. Almost all of the currently used influenza vaccines have been designed to induce antibodies (Abs) against the viral surface glycoproteins, hemagglutinin (HA) in particular. These Abs, detected by HA inhibition (HI) assay, are thought to prevent viral attachment to host cells. The post-vaccination serum HI titer has been widely used as a surrogate marker for vaccine efficacy compliant with European Medicines Agency (EMEA) and Food and Drug Administration (FDA) guidelines. However there is mounting evidence that T cell-mediated immunity (CMI) plays an important role in controlling influenza. Studies in both animal models and humans demonstrate that protection can occur in absence or with very low level of Abs and HI titers, pointing out the role of the CMI in the control of the virus replication and the resolution of the disease. Moreover, CMI has recently been demonstrated to play a pivotal role in cross-protection against drifted and heterologous strains [12–15]. Although most of the work on CMI to date has focused on the conserved epitopes of internal influenza proteins like nucleoprotein (NP) and M protein, recent data demonstrate that the influenza HA molecule also contains class I- and class II-restricted epitopes and that HA-specific T cell responses can be detected after influenza infection or vaccination [16–20].

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Virus-like particle (VLP) influenza vaccines made in plants have proved to be immunogenic in both animal models and humans [21–24]. Plant-based VLP vaccines have the potential to address several of the limitations of currently licensed products including response time and scalability in the event of a pandemic. Furthermore, the plant-based vaccines target wild-type HA sequences in contrast to influenza strains grown in eggs or tissue culture that may have mutated for optimal growth, possibly compromising the effectiveness of the vaccine [25]. Finally, some highly-pathogenic avian influenza (HPAI) strains like H5N1 replicate very poorly in eggs thereby reducing vaccine production capacity.

Herein we described the results from a phase 1–2 randomized clinical trial conducted to assess the safety, tolerability, and immunogenicity of a non-adjuvanted plant-derived quadrivalent VLP influenza (QVLP) vaccine in healthy adults. This QVLP vaccine was well-tolerated and elicited sustained, cross-reactive humoral and cell-mediated immune responses.

2. Methods

2.1. Production of plant-derived HA VLP influenza vaccine

The QVLP vaccine was produced in *Nicotiana benthamiana* using the Agrobacterium infiltration-based transient expression plateform as previously described [21,26]. The HA protein in the VLPs were based on HA sequences of A/California/07/2009 H1N1 (A/H1N1 Cal), A/Victoria/361/11 H3N2 (A/H3N2 Vic), B/Brisbane/60/08 (B/Bris, Victoria lineage) or B/Wisconsin/1/10 (B/Wis, Yamagata lineage) influenza strains according to the recommendations of the World Health Organization for the 2013–2014 North Hemisphere influenza season. Drug Substances for each strain were combined into a quadrivalent Drug Product. Final doses were based on HA content. Each dose was administered in a volume of 0.5 ml in the deltoid muscle of the non-dominant arm.

2.2. Study design and objectives

Eligible male and female subjects between 18 and 49 years of age were enrolled for this phase 1–2 randomized, double-blind, placebo-controlled, dose-ranging clinical trial (NCT01991587 at ClinicalTrials.gov) conducted in the United States between May 2013 and February 2014. The study was carried out in accordance with the Declaration of Helsinki and the principles of Good Clinical Practices and was approved by the site's Ethics Review Board and by the Center for Biologics Evaluation and Research (CBER). Criteria of exclusion were detailed in the Suppl. Table 1. The primary objectives were to evaluate safety and tolerability of a single dose of the QVLP vaccine administered intramuscularly (IM) as well as the immunogenicity of the vaccine as measured by the HI antibody assay. Secondary and exploratory objectives focused on cross-reactivity of the HI antibody response, neutralisation of homologous and heterologous strains in the microneutralization (MN) assay and CD4 T cell responses against homologous and heterologous strains. Antibody responses (e.g.: IgG, IgE) to plant glycans were also measured by the ImmunoCap test (Pharmacia & Upjohn, Uppsala, Sweden).

2.2.1. Study procedures

The Phase 1 portion of this study used dose escalation with slow enrolment (staggered cohorts) for the 3 dose levels (3 μg, 9 μg, and 15 μg VLP per strain) with a placebo-controlled group. In the first Cohort, 13 subjects were randomized to receive the lowest QVLP dose (3 μg of each strain: $n = 10$) or placebo (phosphate buffer saline pH 7.4: 100 mM $NaKPO_4$, 150 mM NaCl, 0.01% Tween-80, $n = 3$). Once the 7-day safety data had been reviewed by the Data and Safety Monitoring Board (DSMB) the second cohort of 13 subjects was randomized to receive the medium dose of QVLP (9 μg of each strain: $n = 10$) or placebo ($n = 3$). Again, DSMB review of the 7-day safety data permitted randomization of the third cohort to receive either the high dose of QVLP (15 μg of each strain, $n = 10$) or placebo ($n = 4$). Upon satisfactory DSMB review the 7-day safety data for the third cohort, the Phase 2 portion of the study was initiated during which 80 subjects were randomised (1:1:1:1) to receive one of three QVLP vaccine doses or placebo. Blood samples for hematology and biochemistry and urine (routine & microscopic) were collected at screening, D3 and D201. Serum pregnancy tests (for female subjects) were performed at screening, D0, D21 and D201. Serum samples were obtained at D0, D21 and D201for HI and MN assays and measurement of antibodies against plant glycans. Anti-coagulated (EDTA) whole blood samples were collected in a subset of 10 subjects per group for analysis of T cell responses at D0, D21 and D201.

2.2.2. Safety/reactogenicity assessments

Subjects were observed for 30 min after vaccination for immediate reactions and were provided with diary cards to record solicited local or systemic reactions that occurred up to seven days after vaccination. Solicited local reactions included pain, swelling and redness (or erythema). Solicited systemic reactions included fever (≥38 °C), headache, muscle aches, joint aches, fatigue, chills, feeling of general discomfort or uneasiness and swelling (axilla, groin, neck, and/or chest). The severity of reported symptoms was assessed as mild, moderate or severe. Unsolicited adverse events (AEs) were collected from D0 through D21. All serious adverse events (SAEs), new onset of chronic disease (NOCD) and AEs leading to study withdrawal were collected throughout the study. Causality of all unsolicited AEs/SAEs/NOCDs was evaluated by the investigator and reported as definitely not related, probably not related, possibly related, probably related or definitely related.

2.2.3. HI assay

The HI assay was performed on serum samples as previously described according to WHO recommendations [23,27]. The homologous antigens (Ag) tested were A/H1N1 Cal propagated in Madin-Darby (Southern Research Institute), A/Texas/50/2012 H3N2 (A/H3N2 Tx, NYMCX-223 A, National Institute for Biological Standards and Control, NIBSC) a virus antigenically like the cell-propagated virus A/H3N2 Vic, B/Bris (NYMC BX-35, NIBSC) and B/Wis (NIBSC #12/110). The heterologous Ag tested were A/Brisbane/59/2007 H1N1 (A/H1N1 Bris, IVR-148, NIBSC #08/100), A/Uruguay/716/2007 H3N2 (A/H3N2 Uru, NYMC-175C, NIBSC #08/278), B/Malaysia/2506/2004 (B/Mal, NIBSC #08/184) and B/Massachusetts/02/2012 (B/Mass, NIBSC #13/152). Serum titers are expressed as the reciprocal of the highest dilution that showed complete inhibition of hemagglutination. Although some controversy subsists, HI titer ≥1:40 remains the reference standard indicative of seroprotection [28–30]. Seroconversion rate (SCR), seroprotection rate (SPR) and geometric mean fold rise (GMFR) were defined according to regulatory criteria [31] and were compared to the European Agency for the Evaluation of Medicinal Products' CHMP criteria i.e. SCR ≥ 40%, SPR ≥ 70%, GMFR ≥ 2.5 for healthy adults.

2.2.4. MN assay

MN antibody titers against the homologous strains A/H1N1 Cal, A/H3N2 Vic, B/Bris, B/Wis and the heterologous strain B/Mass were measured in serum at D0 and D21 according to the World Health Organisation guideline for the serologic diagnosis of influenza [27]. MN titers are expressed as the reciprocal of the highest dilution that showed complete neutralization of input virus. Sera that tested negative at a dilution of 1:10 were assigned a titer of 5 for statistical analysis. Samples were analyzed in duplicates and repeats were performed when acceptance criteria for the assay were not met.

2.2.5. Ex vivo T cell re-stimulation assay

Peripheral blood mononuclear cells (PBMCs) were collected at D0, D21 and D201 after vaccination. Cells were prepared and cryopreserved in liquid nitrogen until quickly thawed and prepared for the assays as