

SAFETY AND IMMUNOGENICTY OF SEASONAL INFLUENZA TRIVALENT VLP VACCINE IN NEW ZEALAND WHITE RABBITS

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ABSTRACT

Virus-like particles (VLPs) are similar in structure to a virus but lack the genetic material required for viral replication. Because they more closely match an individual viral strain, VLPs can trigger a robust immune response without the need for adjuvant, and pose no risk of infection. The purpose of this study was to evaluate the potential toxicity and immunogenicity of Seasonal Trivalent Influenza VLP Vaccine (Vaccine). Five rabbits/sex/group were assigned to one of three treatment groups, and five additional rabbits/sex/group were assigned as recovery animals. Animals were administered 0.5 mL of either control article or Vaccine at 30 or 85 µg HA (hemagglutinin) twice by intramuscular injection two weeks apart. Three days following the second dose, the first 5 animals/sex/group were euthanized and necropsied, and the remaining animals were euthanized and necropsied one month following the second dose. Parameters evaluated included mortality, clinical observations, body weights, Draize observations, food consumption, body temperature, ocular examination, clinical pathology, hemagglutination inhibition (HAI), gross pathology, absolute and relative organ weights, and histopathology. Following the second dose of the vaccine there were no dermal Draize observations in control animals of either sex or in treated females. However, several treated male animals had observations of slight edema and/or erythema at injection sites that were transient in nature but not adverse because of the low severity scores. Additional test article-related findings in treated males and females consisted of increased mean total white blood cell counts, increased absolute monocyte counts, and increased mean plasma fibrinogen. In addition increased iliac lymph node and spleen weights and splenic lymphoid hyperplasia were noted in high dose males, and subacute inflammation was present at the injection site of high dose males and females. All of these effects were related to immune stimulation and non-adverse. Analysis of HAI for anti-Influenza (A/Brisbane 59/07 [H1], A/Brisbane 10/07 [H3], and B/Florida 4/06 [B]) antibodies indicated that treated animals mounted an HAI response to vaccination. Based on these findings the No-Observed-Adverse-Effect Level for the vaccine is 85 µg HA.

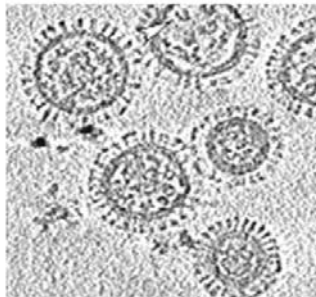
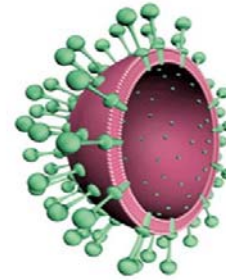
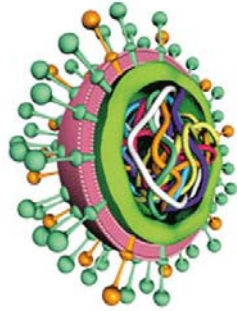
INTRODUCTION

Seasonal influenza is a viral infection that attacks the respiratory tract including the nose, throat and occasionally the lungs. The infection, which usually lasts about a week and is highly contagious, is characterized by fever, headache, muscle aches, cough and sore throat. Each year seasonal influenza infects between three million and five million people worldwide and results in 250,000 to 500,000 deaths. Most of these deaths are associated with complications from pneumonia with the elderly being the most vulnerable. Therefore, there is an urgent need to develop vaccines against influenza sub-types responsible for yearly epidemics that are safe, highly immunogenic, highly efficacious, capable of inducing a broad immune response, and with improved efficacy in infants, children, and older adults.

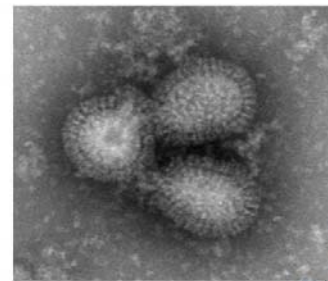
One approach is the use of virus-like particles (VLPs). These are similar in structure to a virus but lack viral nucleic acids required for viral replication, and therefore present no threat of infection to a person being vaccinated. Because they more closely match an individual viral strain, VLPs can trigger a robust immune response without the need for adjuvant. Once injected into the body, VLPs are processed by antigen presenting cells and trigger an immune response sufficient for protection against disease.

The purpose of this study was to evaluate the potential toxicity of Seasonal Trivalent Influenza Vaccine when administered by intramuscular (IM) injection on SD 1 and 15 to New Zealand White rabbits during a 43-day study period. This study was also designed to investigate the reversibility of any treatment-related toxicity during the 28-day no-treatment recovery period. Parameters evaluated included mortality, clinical and cageside observations, body weights, body weight changes, dermal Draize observations, food consumption, body temperature, ocular examinations, selected clinical pathology parameters (clinical chemistry, hematology, and coagulation), hemagglutination inhibition assay (HAI) data, gross pathology, absolute and relative organ weight data, and histopathology.

Influenza VLPs possess the immunogenic surface proteins of the native virus but lack nuclear material and therefore cannot replicate



Virus



VLPs



MATERIALS AND METHODS

Test Animals and Husbandry

New Zealand White rabbits weighing between 1.9 and 2.4 kg and 10-11 months of age were obtained from Harlan Sprague Dawley (Oxford, MI), and acclimated to laboratory conditions for 8 days prior to the first dose. Animals were food fasted on the day of arrival, and given increasing amounts of Certified Global Harlan Teklad Laboratory Diet 2030 for the first two days after arrival and *ad libitum* thereafter. Environmental controls for the animal room were set to maintain a temperature of 16 to 22°C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle. In addition to standard husbandry procedures, animals were provided with toys as environmental enrichment.

Test and Control Articles

Table 1. Test Materials

Name	Lot/Batch No.	Supplier	Purity/ Concentration	Description
Trivalent 08/09 BPL-Treated Seasonal Influenza VLP Vaccine ^a	75508013-2	Novavax, Inc. Rockville, MD	60 µg HA/mL	Translucent, semi-transparent, colorless liquid free of foreign particles
	75508013-4		170 µg HA/mL	
25 mM sodium phosphate/ 0.5 M NaCl	BP04908053		Assumed 100%	Clear, colorless liquid and free of foreign particles

a - Equivalent to Trivalent Seasonal Influenza (A/Brisbane 59/07 + A/Brisbane 10/07 + B/Florida 4/06) VLP Vaccine. BPL = beta-propiolactone.

All test and control articles were provided pre-formulated in vials for single-use dosing. Duplicate vials for stability analysis and dose concentration verification were taken from the test and vehicle/control articles at the time of first and last use.

Experimental Design

Animals were initially accepted into the randomization pool based upon pre-study body weights, physical examinations, and ocular examinations. Males and females were randomized separately, and assigned to study as presented in Table 2.

Table 2. Study Design

Group	Treatment	Dose Level (μg HA/ monovalent VLP)	Dose Volume (mL/animal)
1	Control Article ^a	0	0.5
2	Test Article ^b	30	0.5
3	Test Article ^b	85	0.5

a - 25 mM sodium phosphate/0.5 M NaCl

b - Trivalent 08/09 BPL-Treated Seasonal Influenza VLP Vaccine

Dosing formulations were maintained on wet ice throughout dosing and each vial was inverted 7-10 times prior to injection. Animals were dosed via intramuscular injection (1-cc syringe with a 25-gauge, 5/8" needle) into the right hind limb on SD 1 and into the left hind limb on SD 15 at a dose volume of 0.5 mL; the dose volume was not adjusted for body weight. The injection site was shaved and marked prior to the first dose and was reshaved and remarked as necessary to consistently visualize the dose site.

Animals were observed as shown in Table 3.

Table 3. Animal Observations/Measurements

Procedure	Frequency
Cageside Observations	\geq twice daily and on SD 1 and 15 within 4 ± 0.5 h of the time of dosing
Physical Examinations	4-8 hours following dosing on SD 1 and 15; weekly thereafter
Body Weights	On SD 1 and 15, weekly thereafter, on the day prior to necropsy (unfasted) and at termination (fasted)
Food Consumption	Daily except when interrupted for study-related events
Ophthalmologic Examinations	Prior to necropsy (all surviving animals at each interval)
Body Temperature	The day prior to dosing, within 4 ± 0.5 h of dosing on SD 1 and 15, and daily for the five days after each dose (all surviving animals at each interval)
Dermal Draize Observations	On SD 1 and 15 prior to dosing, within 4 ± 0.5 h of dosing on SD 1 and 15, and daily for the five days after each dose (all surviving animals at each interval). Sites were not scored again after the 5-day post dose interval.

Cageside observations included observation for mortality, moribundity, general health and signs of toxicity. Physical examinations included evaluation of skin and fur characteristics, injection sites, eye and mucous membranes, respiratory,

circulatory, autonomic and central nervous systems, and somatomotor and behaviour patterns.

Ophthalmologic observations were conducted using indirect ophthalmoscopy and slit-lamp (as needed) following administration of a mydriatic solution.

The injection sites were evaluated according to the Draize scoring scale.

Blood was collected for clinical pathology evaluation and HAI assay as shown in Table 4.

Table 4. Clinical Pathology

Parameter	Chemistry	Hematology	Coagulation	HAI
Collection Day	Prior to the first dose, SD 3, 18, and 43 (all surviving animals at each interval)			Prior to the first dose, SD 15, 29, and 43 (all surviving animals at each interval)
Collection Method	Puncture of the medial auricular artery			
Volume Collected	1.2 mL	1.2 mL	1.8 mL	2 mL (1 mL in each of 2 tubes)
Tubes Used	Serum separator	K ₂ EDTA	Sodium citrate	Serum separator

Termination and Postmortem Procedures

Necropsies were performed on SD 18 (main phase) and SD 43 (recovery phase). A gross necropsy, which included examination of the external surface of the body, all orifices, injection sites, the cranial, thoracic, and abdominal cavities, and their contents, was performed. Organs were weighed as soon as possible after dissection; paired organs were weighed together. Tissues were preserved in 10% neutral buffered formalin (NBF) with the exception of eyes (with optic nerve) and testes (with epididymides) which were preserved in modified Davidson's fixative for 24-72 h and subsequently transferred to 10% NBF. Two bone marrow smears were prepared from the sternum. Protocol-required preserved tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin by AVANZA. The slides from each main and recovery phase animal in Groups 1 and 3 were examined by a board-certified veterinary pathologist.

RESULTS

Stability and Dosage Analyses

The data indicate that the potency for all three monovalent flu strains comprising the vaccine, as measured in $\mu\text{g HA/mL}$, was consistent with the values as reported in the Certificates of Analysis (CoA). The data further indicate that the potency in $\mu\text{g HA/mL}$ of the three monovalent strains was within acceptable limits ($\pm 10\%$). Therefore, the vaccine was considered stable for the duration of the study under the stated conditions of storage and use, and the samples met potency specifications indicating that the correct doses of the vaccines were administered to the study animals.

In addition, the pH, appearance, and osmolarity of both the VLP vaccine and the vehicle/control article were consistent with the values as reported in the respective CoAs.

Animal Disposition and Clinical Observations

Treatment with Trivalent 08/09 BPL-treated Seasonal Influenza VLP Vaccine at doses of 30 or 85 $\mu\text{g HA/dose}$ had no adverse effect on mortality, clinical or cageside observations. Observations of scabbing of the nose and abrasion around the mouth were unrelated to administration of the vaccine because they are frequent observations in laboratory rabbits.

Dermal Draize Observations

Following dosing on SD 1, one or two female rabbits at all dose levels were noted with minimal edema and/or erythema and one male treated with vehicle was noted with minimal edema at injection site 1 that was transient in nature. Because the incidence and severity of observations in vehicle/control article and treated animals was similar, these observations were considered test article-related, but were not adverse. Following the second dose of the vaccine there were no observations in control animals of either sex or in females in Groups 2 and 3. However, several male animals in Groups 2 and 3 had observations of slight edema and/or erythema that were transient in nature. These observations were considered test article-related because they were not present in control animals, but were not adverse because of the low severity scores.

Body Weight, Body Weight Change, and Food Consumption

Treatment with Trivalent 08/09 BPL-treated Seasonal Influenza VLP Vaccine at doses of 30 or 85 µg HA/dose had no adverse effect on absolute body weight gain, body weight change, or food consumption.

Body Temperatures

Treatment with Trivalent 08/09 BPL-treated Seasonal Influenza VLP Vaccine at doses of 30 or 85 µg HA/dose had no effect on body temperatures. Occasional statistical differences were noted, but these findings were not test article-related because there was no consistent temporal relationship to dosing and because there was no effect following the second dose.

Ophthalmology

There were no treatment-related ocular effects following treatment with Trivalent 08/09 BPL-treated Seasonal Influenza VLP Vaccine at doses of 30 or 85 µg HA/dose.

Clinical Pathology

There were no test article-related effects on clinical chemistry parameters. On SD 18, there were test article-related increases in mean total white blood cell (WBC) and absolute monocyte (ABMONO) counts in Groups 2 and 3 males and females but was not adverse because all values were within the historical normal reference range from healthy New Zealand White rabbits.

On SD 3 and 18, there was a test article-related increase in mean plasma fibrinogen (FIB) values in Groups 2 and 3 males and females. This test article effect was not adverse because it is an expected finding consistent with immune stimulation associated with administration of a vaccine test article.

Hemagglutination (HAI) Assay

HAI analysis of serum for anti-Influenza antibodies indicated that all animals in Groups 2 and 3 formed antibodies against all three influenza strains confirming exposure. There was no HAI response noted for animals in the control group.

Macroscopic Findings and Organ Weights

There were not test article-related effects noted the terminal or recovery sacrifice.

At the terminal sacrifice, there were test article-related alterations in iliac lymph node and spleen weights. Absolute and relative weights of the left iliac lymph node were increased for Group 3 males, as were absolute, but not relative weights of the right iliac lymph node. Absolute and relative spleen weights were also increased for Group 3 males. This effect was consistent with immune stimulation associated with administration of a vaccine test article and was considered non-adverse. There were no test article-related organ weights changes at the end of the recovery period.

Histopathology

At the terminal sacrifice, test article-related inflammation occurred at injection site (2) in five of ten Group 3 animals. This effect was transient and considered non-adverse. Test article-related minimal lymphoid hyperplasia of the iliac lymph nodes and occurred in two Group 3 males, and minimal splenic lymphoid hyperplasia occurred in two additional Group 3 males, correlating with increased total WBC and increased lymph node and spleen weights for Group 3. This effect was consistent with immune stimulation associated with administration of a vaccine test article and was considered non-adverse. There were no test article-related microscopic findings following the recovery phase.

CONCLUSIONS

Trivalent 08/09 BPL-Treated Seasonal Influenza VLP Vaccine administered by intramuscular injection to male and female New Zealand White rabbits at doses of 0, 30, or 85 µg HA/monovalent VLP on SD 1 and 15 had no adverse effects on mortality, clinical or cageside observations, body weights, body weight changes, food consumption, body temperature, ocular findings, clinical chemistry, or gross pathology. Following the second dose of the vaccine there were no dermal Draize observations in control animals of either sex or in females in Groups 2 and 3. However, several male animals in Groups 2 and 3 had observations of slight edema and/or erythema that were transient in nature. These observations were considered test article-related because they were not present in control animals but were not adverse because of the low severity.

HAI analysis for anti-Influenza (A/Brisbane 59/07 [H1], A/Brisbane 10/07 [H3], and B/Florida 4/06 [B]) antibodies indicated that all animals in Groups 2 and 3 elicited an HAI response to vaccination, confirming exposure.

Test article-related findings consisted of increases in mean total white blood cell and absolute monocyte counts in Group 2 and 3 males and females on SD 18; increases in mean plasma fibrinogen values in Groups 2 and 3 males and females on SD 3 and 18; increased iliac lymph node and spleen weights for Group 3 males on SD 18; iliac lymph node and splenic lymphoid hyperplasia for Group 3 males on SD 18; and subacute inflammation at injection site (2) for Group 3 animals on SD 18. All of these effects were related to immune stimulation associated with administration of a vaccine test article and were considered non-adverse. There were no test article-related findings following the recovery period indicating reversibility. Therefore, under the conditions of this study, the No-Observed-Adverse-Effect-Level (NOAEL) for the vaccine is 85 µg HA.