



SAFETY AND IMMUNOGENICITY OF AN INHALED DRY-POWDER MEASLES VACCINE

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ABSTRACT

Needle-free aerosol delivery of a dry-powder measles vaccine may provide an effective and low-cost means of immunization of children in developing countries. The advantages over that of the freeze-dried marketed product are that the dry-powder vaccine requires no reconstitution or use of needles for clinical use. Aktiv-Dry has developed an alternative to freeze drying of the measles vaccine that results in preservation of viral potency and particle sizes optimal for alveolar deposition. Therefore, the purpose of this study was to evaluate the safety and immunogenicity of the dry-powder measles vaccine when administered as a single dose to Rhesus monkeys. Two dry powder inhalers in development, PuffHaler[®] and Solovent[™], were utilized, and the animals were dosed once by either nasal delivery or by inhalation delivery. The control groups received the marketed measles vaccine via subcutaneous injection. Animals in the treatment groups received 50 mg of dry powder measles vaccine via PuffHaler[®] inhalation and nasal administration or Solovent[™] inhalation and nasal administration. All vaccinated animals developed protective levels of neutralizing antibodies (>120 mIU/ml). Animals remained healthy throughout the course of the study; no effects on clinical observations, body weights, respiration rates, or breathing patterns were noted. The monkeys were subsequently sent to Johns Hopkins University where they were challenged with live measles virus; neither clinical symptoms nor viremia were observed in animals immunized by inhalation. A subsequent GLP study is underway to evaluate the toxicology and immunogenicity of the dry-powder measles vaccine administered twice to Rhesus monkeys by inhalation using both the PuffHaler[®] and Solovent[™] devices.

INTRODUCTION

Measles is a highly contagious human disease caused by the measles virus (MV), and vaccination programs have dramatically reduced its incidence. However, despite the success of global measles vaccination programs, measles was still responsible for an estimated 345,000 deaths in 2005 (Wolfson et al., 2007), with most of these deaths occurring in developing countries. Measles outbreaks also continue to occur in developed countries that have failed to maintain a high level of population immunity (Moss and Griffin, 2006). Global vaccination coverage is approximately 80% but more than 23 million infants did not receive their first dose of measles-containing vaccine in 2007 (WHO, 2008). Although measles control goals (WHO, 2008) can be achieved with the current vaccination strategies, new measles vaccine formulations that are more easily administered, stable at ambient temperatures, easily transported, and cost effective would be beneficial (Sievers et al, 2008). In addition, safety, disposal, and wastage issues associated with using current lyophilized vaccines that require reconstitution and needles for injection remain a concern (John, 2008).

Aerosol delivery of measles vaccine may provide some or all of the required features mentioned above. A number of clinical studies has been performed using aerosol measles vaccination by nebulizing commercial lyophilized formulations after reconstitution, and this route of administration resulted in equal or better immune responses in children greater than 10 months of age compared with injection (Low et al., 2007). Aerosol delivery of measles vaccine appears to be safe because nearly 4 million children in Mexico were immunized using this route of delivery with no reports of significant adverse events (Valdespino-Gomez et al., 2006). Dilraj et al. (2000) reported that the antibody response in school-age children to Edmonston-Zagreb aerosol vaccine was better after 1 year than the response in children vaccinated subcutaneously. In a follow-up study, the antibody levels and the percentage of children who remained seropositive 6 years later were higher in the group that received vaccine by aerosol than the group that received vaccine by injection (Dilraj et al., 2007).

Rhesus macaques are considered the best experimental model for studying human measles disease and are often used to test novel measles vaccines prior to clinical studies (Wyde, et al., 2001; Auwaerter et al., 1999). Following either natural exposure or challenge with wild-type MV strains, macaque viremia occurs and the infected animals exhibit all the signs of measles commonly seen in humans (Remfry, 1976). Vaccination of macaques by inhalation of an Edmonston-Zagreb-derived measles dry powder vaccine produced by milling was attempted by de Swart et al. (2007). Though measles virus was detected in bronchoalveolar lavage (BAL) samples taken 6 days after vaccination, the virus neutralizing antibody response in these animals was much lower than in animals vaccinated by intramuscular injection. Because vaccination with aerosolized liquid measles vaccine proved effective in a previous study with macaques, it is likely that the limited immune response observed with the dry powder vaccine may have been caused by the aerosol properties of the dry powder formulation or the method of delivery to the lungs (de Swart et al., 2006).

The purpose of this study was to evaluate the general safety and tolerability as well as to characterize the immune response of rhesus macaques to dry powder measles vaccine manufactured by a CO₂-Assisted Nebulization with a Bubble Dryer® process and delivered by inhalation using either PuffHaler® or Solovent™ dry powder inhalers. Secondary endpoints included general safety and tolerability of the measles vaccine dry powder using devices and masks nearly identical to those anticipated to be used in human clinical studies.

MATERIALS AND METHODS

Test Animals and Husbandry

A total of 16 mixed sex, young adult Rhesus monkeys weighing between 2 and 5 kg were obtained from Harlan Sprague Dawley (Indianapolis, IN) and Three Springs Scientific, Inc., Perkasie, PA). Animals were screened for measles and herpes B antibodies prior to arrival, and all animals assigned to study had negative antibody titers.

Animals were acclimated to laboratory conditions for at least 22 days prior to the first dose. During that time, animals had two negative TB tests and were observed for general health and suitability of testing. Certified Global Harlan Teklad Monkey Diet 2055C was provided twice daily and water was provided *ad libitum*. Environmental controls for the animal room were set to maintain a temperature of 18 to 29°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 environmental enrichment consisting of visual contact with other non-human primates, listening to music and/or watching television.

Test and Control Articles

Table 1. Test Materials

Name	Description
Measles dry powder vaccine capsules (for use in Solovent™ device)	Capsules filled with dry powder and packaged in a foil overwrap
Placebo dry powder vaccine capsules (for use in Solovent™ device)	Capsules filled with dry powder and packaged in a foil overwrap
Measles dry powder vaccine blisters (for use in PuffHaler® device)	Aluminum blisters filled with dry powder and packaged in a foil overwrap
Placebo dry powder vaccine blisters (for use in PuffHaler® device)	Aluminum blisters filled with dry powder and packaged in a foil overwrap
SII measles vaccine (lyophilized)	Amber vials with off-white cake powder

The dry powder test articles were provided in a ready-to-use formulation. The control article (SII measles vaccine) was received as a lyophilized powder, and was reconstituted by adding 4.4 mL of Sterile Water for Injection, USP (SWFI). This stock solution (1000 plaque forming units (PFU)/ 0.5mL dose) was diluted with the appropriate amount of SWFI to obtain the Group 2 formulation (100 PFU/0.5 mL dose).

Experimental Design

Animals were acclimated to the mask and nasal delivery administration conditions during the acclimation period. The study design is presented in Table 2.

Table 2. Study Design

Group	Treatment	Nominal Dose	Number of Animals (Males or Females)
1	Control 1: 1000 PFU SII measles vaccine (SC 1000)	1000 PFU measles virus	2
2	Control 2: 100 PFU SII measles vaccine (SC 100)	100 PFU measles virus	2
3	PuffHaler [®] pulmonary administration (P-P)	50 mg dry powder	3
4	PuffHaler [®] intranasal administration (IN-P)	50 mg dry powder	3
5	Solvent [™] pulmonary administration (P-S)	50 mg dry powder	3
6	Solvent [™] intranasal administration (IN-S)	50 mg dry powder	3

PFU - Plaque forming units

All animals were dosed once on Study Day (SD) 1. Animals in Groups 1 and 2 received the SII measles vaccine via 0.5 mL subcutaneous injection to the midscapular region. Animals receiving the dry powder measles vaccine via inhalation were exposed to five puffs of the test article, using either the PuffHaler[®] (Figure 1A) or the Solvent[™] (Figure 1B) device, with each exposure lasting at least 30 seconds. Animals receiving the dry powder measles vaccine by nasal delivery were exposed to 5 actuations of the respective device directly into the nostril, alternating left and right nostril on each actuation.

Figure 1A. Assembled PuffHaler® Device

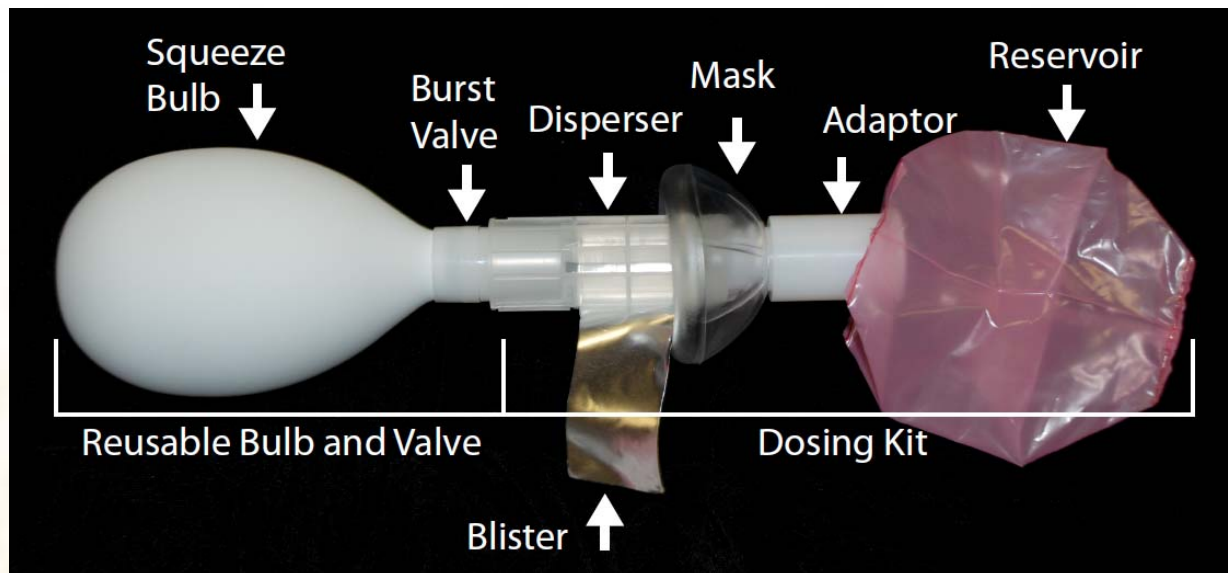


Figure 1B. Assembled Solovent™ Device



Animals were observed for mortality, moribundity, general health and signs of toxicity twice daily. Body weights were collected prior to dosing and at termination. The respiration rate was determined prior to and following the completion of dosing. Breathing patterns were observed during the dosing period.

On SD 6 animals were anesthetized and placed in dorsal recumbancy. Using a swab, the oropharyngeal tonsils of each animal were swabbed, and the swabs were sent to the CDC for analysis. Following swabbing, the mouth was opened using a laryngoscope to expose the glottis and tracheal pathway. To minimize coughing, 0.1 mL of 2% lidocaine was administered topically to the region. A size 3-10 French catheter was advanced until lodged into a subsegmental bronchus. Approximately 3 to 5 mL of saline was infused, the chest area was massaged, and, while massaging, the saline was gently aspirated into a syringe. The procedure was repeated three times and the recovered lavage fluid was placed into appropriately sized tubes and centrifuged at 300g for 15 minutes at 2-8°C. The supernatant was aliquoted into 1-mL or 5-mL portions, and the supernatant and cell pellets were then frozen at $-75 \pm 15^{\circ}\text{C}$ and then sent to the CDC on dry ice for analysis.

Blood samples (each approximately 5 mL) were collected from the femoral vein of each animal prior to dosing on SD 1 and on SD 14, 28, 42, 56, 70, 84, and 98 into tubes containing sodium heparin. Following collection, the blood samples were labeled and sent at ambient temperature on the same day to Johns Hopkins University (JHU).

Following the final blood collection on SD 98, all animals were returned to the stock colony and were subsequently transferred to JHU. All animals were challenged with wild-type measles virus 14-16 months following vaccination. Viral load after challenge was quantitated by RT-PCR.

RESULTS

Observations

The animals were healthy throughout the duration of the study; no significant observations were noted.

Body Weights

Administration of the measles dry powder vaccine had no effect on body weights.

Respiration

The majority of animals had fewer breaths/minute after dosing, including control animals, and no abnormal breathing patterns were noted following administration.

Delivery of the dry powder vaccine to the lung

As expected, no measles RNA was detected in the BAL or tonsil swab samples from animals receiving the vaccine by subcutaneous injection (Table 3). Two of three animals receiving the dose by PuffHaler® inhalation and all of the animals in the PuffHaler® intranasal group had very low levels of measles RNA detected on the tonsil swab samples. All of the animals receiving the vaccine by inhalation had between 500 and 5000 copies of measles RNA in the cell pellets from the BAL. The animals that received the vaccine by intranasal administration had lower levels of measles RNA in the cell pellets from the BAL. All three that were vaccinated intranasally with the Solovent™ device had detectable measles RNA, compared to only one of three vaccinated with the PuffHaler® device (Table 3). None of the supernatants from the BAL samples contained detectable levels of measles RNA (data not shown). Detection of measles RNA in the cell pellets on SD 6 suggests that vaccine virus was actively replicating in these cells and shows that vaccine virus was successfully deposited in the lung following intranasal or pulmonary administration. However, the pulmonary administration appeared to be more efficient regardless of the device used.

Table 3. Detection of RNA from MV in Tonsil Swabs and Bronchoalveolar Lavage Samples from Rhesus Monkeys Receiving Dry Powder Measles Vaccine

Group Number ^a	Vaccine ^a	Sex	Tonsil Swab		BAL ^d Pellet	
			PCR ^b	Copy # ^c	PCR ^a	Copy # ^c
1	SC1000	Male	negative	nd	negative	nd
		Female	negative	nd	negative	nd
2	SC100	Male	negative	nd	negative	nd
		Female	negative	nd	negative	nd
3	P-P	Male	positive	18	positive	678
		Male	negative	nd	positive	549
		Female	positive	24	positive	5280
4	IN-P	Male	positive	245	negative	nd
		Female	positive	86	positive	37
		Female	positive	127	negative	nd
5	P-S	Male	negative	nd	positive	1800
		Male	negative	nd	positive	1110
		Female	negative	nd	positive	488
6	IN-S	Male	negative	nd	positive	326
		Male	negative	nd	positive	71
		Female	negative	nd	positive	72

a - Group number and vaccine as described in Materials and Methods

b - Results from real time RT-PCR assay to detect RNA from measles virus

c - Number of copies of measles RNA/sample based on results from real time RT-PCR assay

d - BAL=bronchoalveolar lavage

nd=not determined

Immune response to dry powder measles vaccine in rhesus macaques

Serum samples from the vaccinated animals were tested for IgG and IgM specific for MV. All of the animals except one in the group receiving the standard dose of MV by subcutaneous injection were seronegative on SD 1 (Table 4). This one animal had detectable IgG on SD 1 and failed to mount a strong IgM response suggesting previous exposure to MV. This animal was excluded from subsequent analysis. All of the animals receiving the vaccine by pulmonary administration and in the Solovent™-intranasal group had detectable MV-specific IgM at Week 4

indicating a primary immune response. IgM was not detected in all of the animals in the groups receiving the low dose of MV by subcutaneous injection and the PuffHaler™-intranasal group (Table 4). It is possible that IgM was present in these animals but at levels below the limits of detection of the enzyme immunoassay (EIA). By Week 8, all of the animals developed a MV-specific IgG response except for two in the PuffHaler® intranasal group (Table 4). Amounts of IgG antibody were highest in the 2 groups of monkeys vaccinated by the pulmonary route. The avidity of the MV-specific IgG matured over time in all groups.

Table 4. Detection of Measles IgG and IgM in Serum Samples from Rhesus Monkeys Receiving Dry Powder Measles Vaccine

Group; Number ^a	Vaccine ^a	MV IgG EIA Result ^b					MV IgM Result ^b	
		Week 0	Week 2	Week 4	Week 6	Week 8	SD 1	Week 4
1	SC1000	I	P	P	P	P	N	N
		N	N	P	P	P	N	P
2	SC100	N	N	P	N	P	N	N
		N	N	I	N	P	N	P
3	P-P	N	P	P	P	P	N	P
		N	N	P	P	P	N	P
		N	I	P	P	P	N	P
4	IN-P	N	N	I	P	P	N	N
		N	N	N	N	I	N	I
		N	N	I	I	N	N	N
5	P-S	N	P	P	P	P	N	P
		N	P	I	P	P	N	P
		N	P	P	P	P	N	P
6	IN-S	N	N	P	P	I	N	P
		N	N	I	P	P	N	P
		N	I	P	P	P	N	P

a - Group number and vaccine as described in Materials and Methods

b - Results from CDC IgM or IgM EIA assays. P=positive, N=negative, I=indeterminate

Levels of neutralizing antibodies in excess of 120 mIU/mL are considered protective. Neutralizing antibodies to MV were detected in all of the animals in the study by four weeks after vaccination, and were also present in all animals at Week 14 (Table 5). The highest titers of neutralizing antibodies were present in both groups of animals receiving the vaccine by the pulmonary route, though five of six animals in the intranasal groups also had titers of greater than 120 mIU/mL in Week 14 (Table 5).

T cell responses measured by IFN- γ ELISPOT assays on PBMCs stimulated with peptides from the Hemagglutinin, Fusion and Nucleoproteins proteins showed responses to all 3 proteins within 2 weeks after immunization. These responses were highest in the monkeys that had received the vaccine by the pulmonary route.

Table 5. Detection of Measles Neutralizing Antibodies in Serum Samples from Rhesus Monkeys Receiving Dry Powder Measles Vaccine

Number ^a	Vaccine ^a	Sex	Plaque Neutralization Titer ^b				
			Week 0	Week 4	Week 8	Week 10	Week 14
1	SC1000	Male	32	2004	10913	1135	706
		Female	<8	245	1853	435	316
2	SC100	Male	8	50	961	100	93
		Female	<8	359	1012	373	304
3	P-P	Male	<8	892	7074	8865	8440
		Male	<8	155	683	956	825
		Female	8	1430	6891	1575	1549
4	IN-P	Male	<8	191	1110	273	189
		Female	8	62	948	93	105
		Female	8	416	1443	534	446
5	P-S	Male	8	1209	13643	2491	1627
		Male	8	284	5440	1117	1063
		Female	32	834	23688	7650	5249
6	IN-S	Male	32	2267	12122	1004	772
		Male	8	72	5733	306	256
		Female	32	122	12609	1154	2132

a - Group number and vaccine as described in Materials and Methods

b - Titers are given in mIU/mL. A titer of 120 mIU/mL is considered protective.

Challenge

No rash was observed in any of the vaccinated animals, but three unvaccinated control monkeys developed rashes following challenge. Infectious measles virus in the blood was detected in only 2 of the 16 vaccinated monkeys seven days following challenge; one animal in the IN-P group and one animal in the low dose sc group showed a low titer of viremia. In contrast, all unvaccinated animals had high levels of infectious virus through Day 10 post-challenge.

CONCLUSIONS

The dry powder vaccine given by either the nasal or pulmonary inhalation route was well tolerated; no effect on clinical observations, body weights, respiration rates or breathing patterns were noted. Protective immune responses were induced in Rhesus monkeys that are at least equivalent to the responses induced by subcutaneous injection.

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