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A Novel Nanoemulsion Adjuvant Enhancing the Immune Response to β-propiolactone Inactivated Influenza Virus Using a Nasal Route in a Ferret Model

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ABSTRACT

Background: Nanoemulsions (NE) are oil-in-water emulsions composed of high-energy nanometer-sized droplets that promote immunity by enhancing uptake of antigens into dendritic cells. A specific NE made of pharmaceutically approved ingredients was investigated as an adjuvant for a nasally administered β-propiolactone (βPL)-inactivated influenza vaccine.

Methods: Naïve ferrets were immunized intranasally with βPL-inactivated influenza A/Wisconsin/67/05 (H3N2) virus with or without nanoemulsion. Ferrets received 2 doses on days 0 and 28. Sera were collected on days 27 and 48 to test for neutralizing antibodies using the hemagglutination inhibition (HAI) assay. Protection was assessed by challenging the ferrets with 10^6 EID_{50} (egg infectious dose) Wisconsin virus on day 49.

Results: The NE-adjuvanted vaccine elicited a more robust immune response with 75% seroconversion and a geometrical mean titer (GMT) of 959 following a single dose of 7.5 µg hemagglutinin (HA) compared to 25% seroconversion and a GMT of 36 without the nanoemulsion. Administration of a second dose resulted in 100% seroconversion and GMT of 3470 in the NE-adjuvanted vaccine group with no improvement in the non-adjuvanted arm. Fluvirin® administered intramuscularly had no increase in Wisconsin HAI titers over background. In challenge studies the NE-adjuvanted vaccine eliminated virus in the ferret nasal washings compared with an average titer of 10^4 - 10^5 EID50 in the non-adjuvanted vaccine.

Conclusions: The use of NE as an adjuvant for βPL-inactivated influenza virus results in a robust serum immune response in naïve ferrets following intranasal immunization. This makes this technology ideal for adjuvanting current seasonal and pandemic influenza vaccines for intranasal delivery.

BACKGROUND

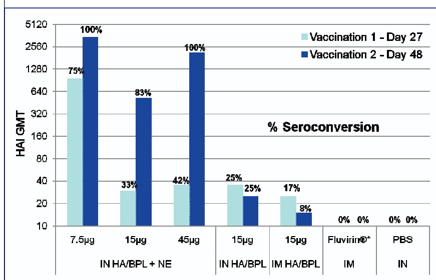
Nanoemulsion-based intranasal vaccines offer several advantages over traditional vaccines. These advantages include antigen-sparing properties, needle-free, painless delivery, and stabilization of the antigen at room temperature. This eliminates the need for cold chain and increases vaccine shelf-life (Makidon et al, *PLoS One*, 13 Aug 2008). These attributes are especially important in remote areas in developing countries.

Nanoemulsion-based vaccines administered intranasally have been shown to provide protective immunity for a number of antigens in animal models: influenza virus in mice (Myc et al, *Vaccine*, Sep 2003), recombinant anthrax protective antigen (Bielinska et al., *Infection and Immunity*, Aug 2007), killed vaccinia virus (Bielinska et al, *Clinical and Vaccine Immunology*, Feb 2008), recombinant HIV gp120 (Bielinska et al, *Aids Research and Human Retroviruses*, Feb 2008); and hepatitis B surface antigen (Makidon et al., *PLoS One*, 13 Aug 2008).

Currently, there are no adjuvants approved for influenza vaccines in the United States. More effective influenza vaccines are needed since current influenza vaccines are sub-optimal. Given this need, we assessed the effectiveness of a NE-based influenza vaccine in ferrets.

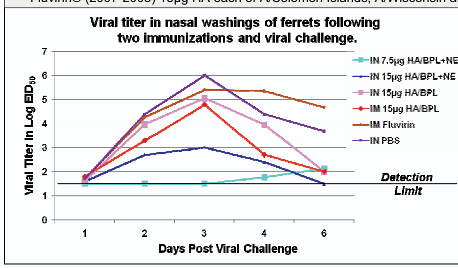
RESULTS

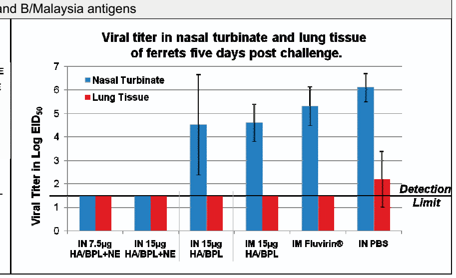
Geometric mean hemagglutination inhibition titers following intranasal nanoemulsion-adjuvanted influenza vaccination in ferrets.



Vaccine	Wisconsin HA (µg)	Route	Dose 1 - Day 27			Dose 2 - Day 48		
			% Sero-Conversion	GMT	Range	% Sero-Conversion	GMT	HAI Range
HA/βPL + NE	7.5	IN	75	959	10-10240	100	3470	1920-7680
HA/βPL + NE	15	IN	33.3	30	10-320	83.3	528	15-3840
HA/βPL + NE	45	IN	41.7	36	10-640	100	2134	320-3840
HA/βPL	15	IN	25	36	10-2560	25	25	10-1280
HA/βPL	15	IM	16.7	25	10-5120	8.3	15	10-1280
Fluvirin®*	15	IM	0	10	10	0	10	10
PBS Control	0	IM	0	10	10	0	10	10

IN= intranasal
IM = intramuscular
HA/BPL = β-propiolactone-inactivated Wisconsin virus (hemagglutinin)
NE = Nanoemulsion
*Fluvirin® (2007-2008) 15µg HA each of A/Solomon Islands, A/Wisconsin and B/Malaysia antigens



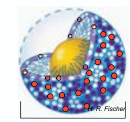


CONCLUSIONS

- The nanoemulsion-adjuvanted vaccine significantly enhanced the immune response of β-propiolactone-inactivated Wisconsin virus following a *single* intranasal dose in naïve ferrets.
- The nanoemulsion-adjuvanted vaccine was antigen sparing and protective against subsequent challenge in ferrets.
- This adjuvant enhances the immune response to different antigens in animal models.
- Given its needle-free application, room temperature stability, antigen sparing properties and the robustness of the immune response this adjuvant could provide a new paradigm for vaccinology.

METHODS

Nanoemulsion-adjuvanted vaccine droplet



Nanoemulsion: The NE adjuvant is an oil-in-water emulsion manufactured from ingredients that are Generally Recognized As Safe (GRAS) with a cationic detergent (cetylpyridinium chloride, CPC) proven safe for human use. The emulsion is formed from highly purified oil, ethanol, polysorbate and water. The average NE droplet size is 400 nm as measured by dynamic light scattering using a Malvern Zetasizer Nano3600 (Malvern Instruments Ltd., UK).

Influenza vaccine preparation: Influenza strain A/Wisconsin/67/05 x A/PR/8/34 X161B was obtained from the CDC. The virus was grown in specific pathogen-free embryonating chicken eggs. The allantoic fluid was clarified by low speed centrifugation, and the virus was concentrated and purified by a 15-60% sucrose gradient centrifugation (120,000 g for 70 minutes). The virus was suspended in PBS and inactivated using β-propiolactone (β-PL, 0.05%v/v) for 16 hours at 4ºC followed by heat degradation of the β-PL at 37ºC for 2 hours. Wisconsin antigen concentration was determined with serial radial immune diffusion assay (SRID) using standard HA antigens and antibodies obtained from the National Institute for Biological Standards and Control (NIBSC), UK. Vaccine preparations were assayed on MDCK cells for absence of live virus. The limit of detection of this assay was ~1.1 pfu.

Nanoemulsion was mixed with different amounts of β-PL-inactivated influenza virus (7.5, 15 & 45 µg HA). Control arms included β-PL-inactivated virus and Fluvirin® commercial vaccine administered IN and IM and IN PBS. Fluvirin® contained 15 µg each of A/Solomon Islands, A/Wisconsin and B/Malaysia antigens (2007-2008 formulation).

Ferrets: Five to eight month-old, influenza-naïve, castrated male Fitch ferrets (*Mustela putorius furo*) were purchased from Triple F Farms (Sayre, PA). Twelve ferrets were vaccinated per arm.

Vaccination schedule: Ferrets received a vaccine dose on days 0 and 28. Blood was collected for HAI assay on days 27 (post-dose 1) and 48 (post-dose 2).

Hemagglutination inhibition assay: HAI assay was performed in duplicate on day 27 and day 48. GMTs were determined from the average of the results.

Post-vaccination challenge: Ferrets were challenged intranasally on day 49 with 1×10^6 EID_{50} A/Wisconsin/67/2005. Following challenge, ferrets were examined daily for signs of disease. No clinical signs were detected in any of the ferret arms following challenge with A/Wisconsin/67/2005 virus. Nasal washes were collected from the ferrets on days 1, 2, 3, 4 & 6 post-challenge for determination of viral load. Four ferrets from each arm were sacrificed on day 5 post-challenge to evaluate viral load in nasal turbinates and lungs. Viral load titers were determined using egg infectious dose 50 (EID_{50}).

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