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Evaluation of a candidate live attenuated influenza vaccine prepared in Changchun BCHT (China) for safety and efficacy in ferrets

Zhenwei Shen [b,1], Richard A. Bowen [c], Peng Ge [b], Jinfei Yu [b], Yanjie Shen [b], Wei Kong [a], Chunlai Jiang [a], Jinchang Wu [a], Changlin Zhu [b], Yanjun Xu [b], Wei Wei [b], Larisa Rudenko [d], Irina Kiseleva [d], Fei Xu [a,*]

[a] School of Life Sciences, Jilin University, Changchun, PR China
[b] Changchun BCHT Biotechnology Co., Changchun, PR China
[c] Department of Biomedical Sciences, Colorado State University, Fort Collins, United States
[d] Department of Virology, Institute of Experimental Medicine, 12 Acad. Pavlov Street, Saint Petersburg 197376, Russia

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ABSTRACT

We evaluated the safety and efficacy of a live attenuated influenza vaccine (LAIV) product in ferrets. The BCHT LAIV product was significantly less virulent than wild-type H1N1 virus, when evaluated by comparing virus shedding and histopathologic lesions. The data indicated strong evidence for an attenuated phenotype of LAIV. Furthermore, the vaccine induced robust humoral immune responses in seronegative ferrets, and protected ferrets against development of fever, weight loss and turbinate inflammatory lesions after challenging with H3N2 wide-type influenza virus. Thus, the BCHT LAIV product was safe in healthy seronegative ferrets and protected ferrets against infection of H3N2 influenza virus.

1. Introduction

In the event of pandemic influenza threat, World Health Organization (WHO) launched the Global Pandemic Influenza Action Plan to increase vaccine supply [1]. Live attenuated influenza vaccines (LAIV) have been used in Russia in adults and children ($\geqslant$3 years old) for nearly 40 years, and proven to be highly efficacious [2–5]. These vaccines may have advantages over inactivated vaccines due to their significantly higher yield, simple production process, needle-free delivery and ability to provide a broader and long-lasting protective immune response [6,7]. To date, WHO has transferred the LAIV production technology to Government Pharmaceutical Organization (GPO) in Thailand, and the Serum Institute of India (SII) [8].

In February 2012, WHO granted Changchun BCHT Biotechnology Co. (BCHT) a sub-license for the development, manufacture and sale of LAIV. Subsequently, BCHT developed the production process of trivalent LAIV based on Russian technology. The virus strains of trivalent LAIV were supplied by WHO, and prepared by the classical reassortment method between wild-type influenza virus strains and master donor virus (MDV) strains, which have the property of attenuation (*att*), temperature-sensitive (*ts*), and cold-adaptation (*ca*). MDVs of A/Leningrad/134/17/57 (H2N2) and B/USSR/60/69 were used for the generation of the Type A (H1N1 and H3N2) and Type B vaccine strains, respectively. The hemagglutinin (HA) and neuraminidase (NA) genes of LAIV strains originate from the wild-type virus recommended by WHO for the coming influenza season, providing specific immune responses. The other six internal genes of LAIV strains come from MDV, providing the *att*, *ts*, and *ca* properties of vaccine reassortant [9–11].

Many studies indicated that monovalent live attenuated pandemic or avian influenza vaccines were safe, immunogenic, and efficacious in ferrets [12–14]. The objective of this preclinical study in ferrets was to evaluate the safety and efficacy of a seasonal trivalent LAIV produced in BCHT for Investigational New Drug (IND) application of clinical trials in China. Ferrets are good model for influenza virus pathogenesis and manifest similar clinical signs of infection that are observed in humans [15]. Safety was evaluated by inoculating ferrets intranasally with the vaccine and comparing virus shedding and histopathologic lesions with ferrets infected with H1N1pdm (A/California/07/2009) virus that was of great concern recently as a pandemic pathogen of humans. Efficacy was evaluated by characterizing humoral immune responses to vaccination and by comparing the effect of vaccination versus placebo administration on virus shedding and histopathologic changes after challenging with a H3N2 (A/Perth/16/2009) subtype influenza A virus.

* Corresponding author.
E-mail address: feixu@jlu.edu.cn (F. Xu).
[1] Current address: Institute of Immunology, Academy of Translational Medicine, First Hospital, Jilin University, Changchun 130061, PR China.

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2. Materials and methods

2.1. Preparation of BCHT trivalent LAIV and wild-type virus

The BCHT live attenuated influenza vaccine strains used for the preparation of the trivalent influenza vaccine were antigenically similar to A/California/07/09 (H1N1), A/Perth/16/09(H3N2) and B/Brisbane/06/09 according to the WHO recommendations for the 2011–12 Northern Hemisphere influenza season (http://www.who.int/influenza/vaccines/virus/2011_12north/en/). Influenza 6:2 reassortant vaccine strains were prepared by the Institute of Experiment Medicine (IEM), Russia and received from WHO. Clarified monovalent virus pools were prepared by inoculating 10-day-old embryonated hen eggs with the working seed virus followed by clarification, ultrafiltration, diafiltration, and sterile filtration of the harvested allantoic fluid. Trivalent vaccine preparations were produced by lyophilizing mixtures of the three monovalent virus bulks with stabilizers.

Each vial of trivalent LAIV powder was reconstituted in 0.2 mL of sterile water immediately prior to use. The virus titer after reconstitution was $10^{7.8}$, $10^{7.5}$ and $10^{7.3}$ EID_{50}/0.2 mL for H1N1, H3N2, and type B influenza strains, respectively.

A/California/07/2009 (H1N1) pdm and A/Perth/16/2009 (H3N2) influenza A viruses were utilized as wild-type viruses in these studies. These two kinds of virus were propagated in embryonated eggs and virus stocks were collected from infected allantoic fluid. The original virus stocks were obtained from BEI Resources (Manassas, Virginia, USA).

2.2. Animals and vaccinations

All procedures applied to animals were approved by the Institutional Animal Care and Use Committee at Colorado State University (protocol 13-4471A) and every effort was made to minimize stress and suffering.

Three to four month old male ferrets were purchased from Triple F Farms (Sayre, Pennsylvania USA) and animals in that facility were routinely tested negative for Aleutian disease virus. The animals were housed loose in rooms in the Animal Disease Laboratory at Colorado State University. Blood samples were collected from all ferrets prior to use. Sera from ferrets were tested negative (<10) for antibodies to H1N1, H3N2 and type B influenza virus by hemagglutination inhibition (HAI) testing.

Safety evaluation: Nine male ferrets were divided into three groups. Three ferrets of group 1 were inoculated intranasally on day 0 with 0.2 mL of wild-type virus, which contains $10^{6.0}$ pfu (plaque-forming units) of A/California/07/2009 virus. Groups 2 and 3 with three ferrets each were inoculated intranasally on day 0 with 0.2 mL of trivalent LAIV. Body temperature, clinical signs and body weight were recorded daily from day −2 to euthanasia. Nasal secretions and oropharyngeal swabs from ferrets in group 1 and 2 were collected for virus titer detection on day −1, 1 and 3. Samples from group 3 were collected on day −1, 1, 3, 5, 7 and 9. Tissues from group 1 and 2 were collected on day 3 for histopathology evaluation and tissues from group 3 were collected on day 14.

Efficacy evaluation: 16 ferrets were divided into two groups with 8 ferrets each for vaccination and PBS control. On day 0, the trivalent LAIV and PBS control were delivered intranasally (0.1 mL per nostril; 0.2 mL total) under ketamine-xylazine anesthesia. Body temperature, clinical signs and body weight were recorded daily from day −2 to +3 for vaccination, and on day 7, 14, 20, 26, and 27. On days 0 and 20, a blood sample was collected from each ferret and serum was tested by HAI for antibodies to all three monovalent viruses. Virus challenge was performed on day 28 for both groups using A/Perth/16/2009 virus diluted in PBS from thawed stocks. Ferrets were anesthetized with ketamine-xylazine and challenged by instilling 1 mL of the challenge virus (2/3 of total dose) intratracheally and 0.5 mL (1/3 of total dose) intranasally for a total dose of 2×10^6 pfu. Body temperature, clinical signs and body weight were recorded from day −2 to termination. Nasal flushes were performed daily from day 1–4, and ferrets were euthanized on day 4 to collect tissues for histopathology.

2.3. Hemagglutination inhibition (HAI) assays

Hemagglutination inhibition (HAI) assays were used to assess humoral immune responses to immunization using standard recommended techniques [16]. Prior to the assay, sera were treated with receptor destroying enzyme (RDE(II), Denka Seiken Co., Tokyo, Japan), followed by heat inactivation at 56 °C for 30 min. Chicken red blood cells were purchased from Lampire Biologics Laboratories (Pipersville, Pennsylvania, USA) and diluted to 0.25% packed cells for assay. The three monovalent virus used in the assay were the same as those contained in the BCHT vaccine. Control antiserum was obtained from the Centers for Disease Control and Prevention (Atlanta, Georgia, USA).

2.4. Sample collection and plaque assay

Collection of nasal flushings and oral swabs to evaluate virus shedding was conducted using techniques as previously described [17]. On the indicated days, ferrets were lightly anesthetized with ketamine-xylazine and placed in a biosafety cabinet. Oropharyngeal swabs were collected by rotating a polyester-tipped swab in the pharynx of the ferrets, then breaking off the swab in 1 mL of BA-1 medium (minimum essential medium supplemented with 50 mM Tris HCl, pH 7.5, 1% bovine serum albumin, gentamicin [50 mg/L], penicillin [200,000 U/L], streptomycin [50 mg/L] and amphotericin B [2.5 mg/L]). Nasal flushings were collected by dripping 1 mL of BA-1 medium into the nares with the ferret in ventral recumbency. The fluid was sneezed out to collect in a Petri dish, followed by pipetted into a sample vial. Samples of both oropharyngeal and nasal flushing fluid were frozen and stored at −80 °C until assay.

At necropsy, samples of the apical and diaphragmatic lung lobes (approximately 100 mg mass) were excised and stored frozen until assay. Immediately prior to the assay, these samples were homogenized in 1 mL of BA-1 medium using stainless steel balls and a mixer mill (Retsch MM 400) operated at 25 Hz for 5 min.

Ten-fold serial dilutions of swab samples, nasal flushings and homogenized samples of lung were prepared in BA-1 medium and inoculated onto monolayers of MDCK cells (ATCC CCL-34) for the A/Perth/16/2009 virus or MDCK-London (provided by CDC, Atlanta, Georgia) for A/California/07/2009 virus. Cells were grown in 6-well plates and inoculated with a volume of 0.1 mL per well, similar to techniques used previously described [17,18]. Cells were incubated at 37 °C for 45 min after virus inoculation, then overlaid with 2 mL of minimal essential medium containing 0.5% bovine serum albumin and 0.8% agarose, supplemented with 1 μg TPCK trypsin/mL. After incubation for 2 days, the virus assay plates were fixed with formalin and stained with crystal violet to allow visualization and counting of plaques. Virus titers are expressed as pfu per mL for oropharyngeal swab and nasal flushing samples, and pfu/gram for tissue samples.

2.5. Histopathology

Tissues collected at necropsy were fixed immediately by immersion in buffered formalin, then trimmed and submitted to a commercial laboratory for embedding, sectioning and staining

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