Biologicals 41 (2013) 247–253

Contents lists available at SciVerse ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Rapid clearance of intranasally administered DNA from rat tissues

David E. Tabor*, Sachin Mani, Xuan Shen, Xiaomu Chen, Charles Engbers, Scott Jacobson, Rosemary Broome, Jonathan Liu, Dominic Justewicz, Mark S. Galinski

MedImmune, 319 North Bernardo Avenue, Mountain View, CA 93043, USA

ARTICLE INFO

Article history:
Received 25 January 2013
Received in revised form
29 March 2013
Accepted 11 April 2013

Keywords:
MDCK cells
Host DNA
Biodistribution
Clearance
Intranasal administration
Intramuscular administration
Influenza vaccine

ABSTRACT

The cold-adapted (ca) live attenuated influenza vaccine (LAIV) strains are manufactured in embryonated hens' eggs. Recently, a clonal isolate from Madin Darby Canine Kidney (MDCK) cells was derived and characterized to assess its utility as a potential cell substrate for the manufacturing of LAIV [1]. Since MDCK cells are a transformed continuous cell line [2], and low levels of residual cellular components (DNA and protein) are found in the intermediates and final filled vaccine, we sought to characterize the uptake and clearance of MDCK DNA from tissues in order to assess theoretical risks associated with manufacturing LAIV in MDCK cell culture.

In order to address this concern, MDCK DNA uptake and clearance studies were performed in Sprague Dawley rats. DNA extracted from MDCK Master Cell Bank (MCB) cells was administered via an intranasal (IN) or intramuscular (IM) route. Tissue distribution and clearance of MDCK DNA were then examined in fourteen selected tissue types at selected time points post-administration using a quantitative PCR assay specific for canine (SINE) DNA.

Results from these studies demonstrate that the uptake and clearance of MDCK DNA from tissues vary depending on the route of administration. When DNA was administered intranasally, as compared to intramuscularly, detectable DNA levels were lower at all time points. Thus, the intranasal route of vaccine administration appears to reduce potential risk associated with residual host cell DNA that may be present in cell culture produced final vaccine products.

1. Introduction

A cell-culture based influenza vaccine program using Madin Darby Canine Kidney (MDCK) cells as the cell substrate was initiated at MedImmune [3,4] to support pandemic preparedness. Theoretical safety concerns related to the use of continuous cell lines such as MDCK cells have been raised related to their use in vaccine manufacture (VRBPAC, 2008). These concerns are primarily associated with residual cellular components (DNA and protein) in the vaccine drug product [5] and are particularly relevant for live attenuated influenza vaccine (LAIV) products that are not inactivated nor undergo extensive biochemical purification as is the case for the traditional influenza (split) vaccines.

Two strategies have been undertaken to minimize these risks: cell line characterization and vaccine purification processing steps. The MDCK cell line referred to in this report was tested for the absence of specific adventitious agents. In addition, the tumorigenicity and oncogenicity of MDCK cell lysates and cellular DNA in rodent models were assessed and demonstrated to be absent [4].

In addition to cell line characterization, manufacturing and processing controls ensure microbial sterility and absence of intact cells using aseptic manufacturing and filtration controls. A specific aspect of the purification process is to reduce the quantity and size of residual host cell DNA in the vaccine product. Although process steps are in place to reduce the amount of residual DNA, there are still detectable levels present in the final bulk harvest. In the present instance, benzonase digestion has led to a significant decrease in the amount and size of residual MDCK DNA in the vaccine product (~0.1 ng/dose, and median size of 450 bp) [6].

It has been suggested that the theoretical impact of host residual DNA on product safety should consider the vaccine administration route, as the tissue distribution and rate of clearance could vary based on the administration modality [7].

This report compares the tissue distribution and the rate of clearance of MDCK DNA administered between two difference routes of administration: intranasal, which is the intended route of administration for LAIV, and intramuscular, which is the traditional

* Corresponding author. Tel.: +1 650 603 2564; fax: +1 650 603 3564.
E-mail address: tabord@medimmune.com (D.E. Tabor).

1045-1056/$36.00
http://dx.doi.org/10.1016/j.biologicals.2013.04.004

route of administration of most vaccines and has been used to define acceptable level of residual host cell DNA in biological products by regulatory authorities. Studies were completed using a Sprague Dawley rat model, where rats were administered sheared MDCK DNA, similar in nature to the residual host cell DNA in MDCK-produced live attenuated influenza vaccine (LAIV), via two routes of administration: intranasal (IN) or intramuscular (IM). Tissue distribution and rates of clearance were examined over time in fourteen selected organs and tissues using a highly sensitive quantitative PCR assay specific for canine (SINE) DNA.

2. Materials and methods

2.1. Cell line expansion and pellet preparation

SF MDCK MCB 9B9-1E4 Lot# 0141000168 cells were expanded in T-flasks and roller bottles through to passage 16, designated as end of production (EOP) passage level. Media was removed, cell monolayers were rinsed with PBS and subjected to trypsinization. Cells were re-suspended in DPBS, pelleted by centrifugation at 3500 rpm for 30 min at 4 °C and the process repeated. Cell count was determined via Cedex Cell Counter, then a final spin at 3500 rpm for 30 min at 4 °C was performed, the supernatant was decanted, and the cell pellet stored at −80 °C.

2.2. MDCK DNA preparation

Cell pellets were thawed in a 32 °C water bath and then re-suspended in 560 mL DPBS (without Ca^{2+} or Mg^{2+}), resulting in a concentration of $\sim 1.32 \times 10^7$ cells/mL. DNA was purified using a Qiagen QIAamp DNA Blood Maxi Kit Cat No. 51194. DNA quantitations were performed using a Nanodrop ND-1000 spectrophotometer.

Sheared DNA was prepared via sonication to obtain DNA in similar size distribution to final bulk Clinical Trial Material (CTM), sonication runs were performed to shear the extracted MDCK DNA using a sonicator set at 7 W for 14 min.

Sheared and un-sheared DNA preparations were precipitated with isopropanol by adding one-tenth volume of 3 M NaOAc (pH 5.5) (~1/10 V) and ~0.9 volume of isopropanol to the DNA. DNA samples were mixed gently by inversion and placed at 4 °C overnight. DNA was subsequently pelleted by centrifugation at 14,000 rpm for 20 min. DNA Pellets were washed twice with cold 70% ethanol (20 mL/tube each) and allowed to air dry. Dried DNA pellets were re-suspended/rehydrated in 4 mL DPBS to a final approximate concentration of 1.0 mg/mL. The DNA concentration was measured via a Nanodrop ND-1000 spectrophotometer.

2.3. DNA administration to rats, tissue harvest, tissue homogenization

Sprague–Dawley (CD1) rats (male and female, 7–9 weeks of age) were obtained from Charles River Laboratories (CRL), Hollister, CA. For the intramuscular (IM) route group, animals were administered 100 μL of 1 mg/mL MDCK DNA by shaving the right flank and injecting the DNA into the quadriceps of each animal on Day 0 using a 21-gauge needle. For the intranasal (IN) route group, animals were administered 100 μL of 1 mg/mL MDCK DNA by gradually pipetting 50 μL of MDCK DNA into each nostril on Day 0. All rats were lightly anesthetized with 2–3% isoflurane in oxygen for dosing.

Following the administration of DNA, all animals were housed individually in internally ventilated microisolator cages. Food and water were provided ad libitum. Rats scheduled for euthanasia were fasted for approximately 24 h prior to tissue harvest.

Each test group administered MDCK DNA was comprised of two males and two females. At the designated time points post-dosing, animals belonging to each test group were euthanized. Before necropsy, the rats were shaved and then liberally sprayed with DNAZap™ (Ambion) and allowed to soak for 5 min. The dissection boards were also soaked with DNAZap™. Tissues and organs were harvested and processed for DNA extraction and quantitation. A saline-administered control animal, either male or female was also euthanized at the designated time points, to serve as a control for the test groups. Fourteen tissues were collected from each animal unless otherwise specified in the study design. Tissues included: whole blood, stomach, intestines, pancreas, spleen, liver, kidneys, lung and trachea, thymus, tongue, nasal cavity, brain, skin (over the IM injection site) and muscle (at IM injection site).

Whole blood was collected from anesthetized animals (isoflurand/O_2) by cardiac puncture. Blood collection syringes were primed with 50 μL of 0.11 M sodium citrate. Blood was collected into the primed syringe and immediately transferred to an autoclaved microfuge tube containing 50 μL of 0.11 M sodium citrate. The sample was mixed, weighed and placed on wet ice. Immediately following blood collection rats were euthanized by carbon dioxide inhalation and prepared for tissue collection by shaving the entire body, portions of the head and rear legs.

The tissues were removed, rinsed lightly with sterile DPBS and placed in autoclaved polycarbonate vials containing 5/32 inch stainless steel beads. The tissues were weighed and OPTI-MEM buffer was added to each vial to facilitate in the homogenization process. The tissue weight to buffer ratios are listed in Table 1. The tissues were collected in the order: blood, pancreas, spleen, stomach, intestines, kidneys, liver, thymus, lung and trachea, tongue, nasal cavity, skin, muscle and brain except for the IM group where skin and muscle were collected last.

On each day of tissue collection, the saline control rat was processed before the DNA-administered rats. In-between tissue collection from individual rats, the dissection area was cleaned and disinfected with Airocide followed by 70% isopropyl alcohol. Tissues were homogenized by placing the vials with tissues and buffer into an OPS Diagnostics linear motion mixing mill "HT Homogenizer Model #TR930145 (OPS Diagnostics, LLC; Lebanon, New Jersey). Mixing time ranged from 5 to 8 min. Samples vials were placed on wet ice immediately following homogenization and subsequently transferred to freezer vials and stored at −80 °C prior to DNA extraction.

2.4. Extraction and detection of MDCK DNA from tissue homogenates

For a given time course experiment, each sample derived from an individual tissue type (e.g. liver) was processed in parallel to

Table 1
Tissues collected and ratio of tissue weights to buffer (OPTI-MEM) used for homogenization.

Tissue	Tissue buffer ratio (w/w)[a]	Tissue	Tissue buffer ratio (w/w)[a]
Blood	NA[b]	Nasal cavity	1:08
Brain	1:08	Olfactory bulb[c]	1:02
Intestine	1:02	Pancreas	1:08
Kidneys	1:04	Skin	1:08
Liver	1:01	Spleen	1:08
Lung and Trachea	1:04	Stomach	1:04
Muscle	1:08	Thymus	1:08
		Tongue	1:08

[a] w/w, weight tissue to weight buffer.
[b] NA, not applicable.
[c] Olfactory bulb was assessed only in Study 3.