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Evaluation of excipients for enhanced thermal stabilization of a human type 5 adenoviral vector through spray drying

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ABSTRACT

We have produced a thermally stable recombinant human type 5 adenoviral vector (AdHu5) through spray drying with three excipient formulations (L-leucine, lactose/trehalose and mannitol/dextran). Spray drying leads to immobilization of the viral vector which is believed to prevent viral protein unfolding, aggregation and inactivation. The spray dried powders were characterized by scanning electron microscopy, differential scanning calorimetry, Karl Fischer titrations, and X-ray diffraction to identify the effects of temperature and atmospheric moisture on the immobilizing matrix. Thermal stability of the viral vector was confirmed *in vitro* by infection of A549 lung epithelial cells. Mannitol/dextran powders showed the greatest improvement in thermal stability with almost no viral activity loss after storage at 20 °C for 90 days (0.7 ± 0.3 log $TCID_{50}$) which is a significant improvement over the current −80 °C storage protocol. Furthermore, viral activity was retained over short term exposure (72 h) to temperatures as high as 55 °C. Conversely, all powders exhibited activity loss when subjected to moisture due to amplified molecular motion of the matrix. Overall, a straightforward method ideal for the production of thermally stable vaccines has been demonstrated through spray drying AdHu5 with a blend of mannitol and dextran and storing the powder under low humidity conditions.

1. Introduction

Adenovirus-based gene transfer vectors have been increasingly developed as vaccine platforms against both old and newly emerging infections (Lasaro and Ertl, 2009; Majhen et al., 2014; Zhu et al., 2015). However, the real world application of adenoviral vectors, in particular in the developing countries, is limited by their instability when stored or transported at even mild temperatures. Alteration of genetic data within viral genomes for vaccine vector applications results in an increased instability in maintaining infectious function (Amalfitano et al., 1998; Havenga et al., 2006). Storage of these vectors within synthetic vials furthermore accelerates denaturing of proteins and loss of viral infectivity through aggregation. Thus, to maintain function, adenoviral vectors suspended in an aqueous medium require storage at temperatures close to −80 °C to maintain 'cold chain' protocols (Nyberg-Hoffman and Aguilar-Cordova, 1999). This condition is critical for inhibiting molecular movements of the stored adenoviruses, hindering their aggregation else resulting in vector inactivation (Kumru et al., 2014; Rexroad et al., 2006, 2003). Immobilization of viral vectors within cold storage conditions are uneconomical, and potentially infeasible in areas around the globe requiring vaccination the most.

A major goal for both the World Health Organization and Bill & Melinda Gates Foundation is to alleviate cold chain requirements for vaccine storage and distribution (World Health Organization, 2011). Hence, thermal stability, as used in reference to new classes of vaccines, refers to the ability of a viral vector to be stored at elevated temperatures (above −80 °C) for prolonged duration without significant loss of activity. A promising approach capable of increasing thermal stability of labile vectors is through their dispersion within the amorphous phase of a solid matrix, termed as vitrification (Crowe et al., 1997; Rexroad et al., 2003). Vitrification of viral vectors within sugars, polymers, amino acids, surfactants, and other materials has maintained viral activity at storage temperatures above typical cold chain temperatures (Alcock et al., 2010; Amorij et al., 2008; Maa et al., 2004).

Previous studies have dictated the importance of matrix physical and chemical properties on thermal stability (Yu, 2001). The production of a solid matrix is known to greatly hinder the molecular movements of an entrapped adenoviral vector, thus preventing unfolding and aggregation (Ihnat et al., 2005). Selection

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of a purely amorphous matrix may result in a solid with high moisture sensitivity (Hancock and Zografi, 1993) which will reduce stabilization of any dispersed labile biological materials (Ahlneck and Zografi, 1990). Conversely, crystalline structures are moisture-resistant but not optimal for stabilizing dispersed biological materials due to poor incorporation within the matrix. Binary excipient mixtures are a novel consideration for stabilizing viral vectors since they can be used to balance the physical characteristics of a formulation (Couchman, 1978; Penning and St. John Manley, 1996), though no current examples are systematically evaluated within the literature. The work presented here demonstrates the potential viability for semicrystalline powders as stabilizing matrices. As pharmaceutical excipients require regulatory approval for use, this work highlights that it is not necessary to be even more restrictive in excipient selection by not considering crystalline and semicrystalline materials. Furthermore, crystallinity may offer material advantages, as previous publications have demonstrated that crystalline regions can act as physical barriers for molecular movements and water sorption (Bronlund and Paterson, 2004; Mihranyan et al., 2004; Mizuno et al., 1998). The present work evaluates two binary sugars and one amino acid formulation to observe the effects of crystallinity and excipient glass transition temperature (T_g) on adenovirus stabilization.

Several drying processes such as spray drying, freeze drying and foam drying have been employed in recent years for producing dry powder forms of solid viral vector dispersions (Jin et al., 2010; Ohtake et al., 2010; Wong et al., 2007). Spray drying is increasingly preferred since its simple requirements facilitate product scalability (Ré, 1998) and favorable economics. During spray drying, a pressurized gas is used to disperse a liquid feed into small droplets within a drying chamber. Evaporation of heated aqueous droplets results in precipitation of the dissolved solutes and suspended materials. Current research aimed at improving thermal stability for labile biological materials has shown great success with spray drying vaccines ranging from attenuated pathogens to antigen-based formulations (Garmise et al., 2007; Jin et al., 2010; Ohtake et al., 2010; Saluja et al., 2010; Wong et al., 2007). The degree of thermal stabilization varies significantly depending on the dispersed biological material. For example, a spray dried bacillus Calmette-Guérin vaccine formulation with L-leucine demonstrated a minimal activity loss of approximately 2.0 log after 120 days at 25 °C under high moisture protection (Wong et al., 2007). Alternatively, an antigen-based influenza subunit vaccine stabilized in inulin retained considerable immunogenicity for up to three years of storage at 20 °C (Saluja et al., 2010). The variance in stability among spray dried biological materials emphasizes the need for specific evaluation of each vaccine backbone and excipient combination.

Human adenovirus type 5 (AdHu5) has been shown to be an effective vaccine vector for prevention of infectious diseases and has been developed in both liquid buffer and lyophilized forms (Frahm et al., 2012; Smaill et al., 2013). Current limitations to AdHu5 use stem from pre-existing AdHu5 immunity and the lack of a thermally stabilized form. It is estimated that 30–100% of the population, depending on geographical location, have been exposed to AdHu5 and therefore elicit an AdHu5-specific response upon infection (Appaiahgari and Vrati, 2014). The anti-AdHu5 immunity pre-existing in most of the human population poses a potential limitation to the application of AdHu5-vectored vaccines. However, the results from our recent clinical vaccine trial suggest that the potency of AdHu5 vector system is able to diminish the negative effect of a pre-existing immunity (Smaill et al., 2013). Furthermore, AdHu5 vector is particularly amenable to vaccination *via* the respiratory mucosal route against lung infectious diseases and the human respiratory tract has been found to have minimal pre-existing anti-AdHu5 immunity (Richardson et al., 2011). Thus, it is expected for AdHu5-based vaccine to be even more effective when given *via* the respiratory mucosal route versus a parenteral route. In terms of thermal stability, AdHu5 has yet to be developed into a well-stabilized spray dried form. This work extends the possible applications of AdHu5 as a vaccine by producing a more thermally stable vector through spray drying with well-accepted excipients. More specifically, we have evaluated binary sugar and amino acid formulations consisting of semicrystalline and entirely crystalline excipient matrices to observe the effects of crystallinity and T_g on AdHu5 stability. The effects of storage time, temperature and humidity were systematically examined on spray dried vector infectivity for AdHu5, which to the best of our knowledge, has not been reported previously. The purpose of this work is to demonstrate a thermally stable spray dried AdHu5 vector and highlight the physical properties necessary for the best stabilization, which can be used to further the field of dry powder vector development. Future developments with these spray dried powders will focus on their use for inhalation and optimizing excipient ratios for better thermal stability of the labile material. The future use of these spray dried powders in inhalable applications is dependent on a suitable safety assessment, as the effects of administration of the studied excipients within this work to the lungs has not been fully established.

2. Materials and methods

2.1. Chemicals and adenoviral vectors

Anhydrous lactose, D-(+)-trehalose dihydrate, D-mannitol, dextran (M_r 40000 kDa) and L-leucine were all purchased as USP grades from Sigma-Aldrich (Ontario, Canada). Culture media was produced from α-minimum essential medium (prepared in the lab according to protocol by Life Technologies (Ontario, Canada)) with 10% fetal bovine serum and 1% streptomycin/penicillin (Invitrogen; Ontario, Canada). X-Gal stock solution was purchased from EMD Millipore (Ontario, Canada). A recombinant replication-defective human type 5 adenovirus expressing *Escherichia coli* β-galactosidase (AdHu5LacZ) was produced in the vector facility of McMaster Immunology Research Centre as described previously (Xing et al., 1996).

2.2. Spray drying

Spray dried powders were produced using a Büchi Mini Spray Dryer B-290 (Büchi; Switzerland) with 0.7 mm spray nozzle and high performance cyclone. The setup is shown schematically in Fig. 1, consisting of (1) the spray drying nozzle, (2) the drying chamber, (3) the separating cyclone and 4) the collection chamber. The atomizing air was dried using an in-line silica gel desiccant air dryer (McMaster-Carr; Elmhurst, IL) and cleaned using an Aervent® 0.2 μm filter (EMD Millipore; Billerica, MA). Three excipient formulations were produced: (1) L-leucine, (2) 90% lactose and 10% trehalose and (3) 67% mannitol and 33% dextran (all compositions are quoted based on percent by weight). Excipient formulations were dissolved in Milli-Q® water. The AdHu5 vector was stored in a PBS buffer; however, its addition to the excipient solution was negligible, being less than 1/10000th of the spray dried volume. The pH of the solution was 6.5. The formulations used within this work were selected from a number of excipients that have been employed within the pharmaceutical and spray drying industry (Amorij et al., 2008; Ohtake et al., 2010; Vehring, 2008; Wu et al., 2014; Yu, 2001) and can be found in the Inactive Ingredient Database for Approved Drug Products (FDA). The reported formulations (Table 1, in terms of excipients used and