Journal of Controlled Release 204 (2015) 38–50

Contents lists available at ScienceDirect

Journal of Controlled Release

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

A method of lyophilizing vaccines containing aluminum salts into a dry powder without causing particle aggregation or decreasing the immunogenicity following reconstitution

Xinran Li [1], Sachin G. Thakkar [1], Tinashe B. Ruwona, Robert O. Williams III, Zhengrong Cui *

The University of Texas at Austin, College of Pharmacy, Pharmaceutics Division, Austin, TX, United States

ARTICLE INFO

Article history:
Received 22 November 2014
Received in revised form 23 February 2015
Accepted 27 February 2015
Available online 28 February 2015

Keywords:
Thin-film freezing
Lyophilization
Aluminum salts
Antibody responses
Aggregation
Repeated freezing-and-thawing

ABSTRACT

Many currently licensed and commercially available human vaccines contain aluminum salts as vaccine adjuvants. A major limitation with these vaccines is that they must not be exposed to freezing temperatures during transport or storage such that the liquid vaccine freezes, because freezing causes irreversible coagulation that damages the vaccines (e.g., loss of efficacy). Therefore, vaccines that contain aluminum salts as adjuvants are formulated as liquid suspensions and are required to be kept in cold chain (2–8 °C) during transport and storage. Formulating vaccines adjuvanted with aluminum salts into dry powder that can be readily reconstituted before injection may address this limitation. Spray freeze-drying of vaccines with low concentrations of aluminum salts and high concentrations of trehalose alone, or a mixture of sugars and amino acids, as excipients can convert vaccines containing aluminum salts into dry powder, but fails to preserve the particle size and/or immunogenicity of the vaccines. In the present study, using ovalbumin as a model antigen adsorbed onto aluminum hydroxide or aluminum phosphate, a commercially available tetanus toxoid vaccine adjuvanted with potassium alum, a human hepatitis B vaccine adjuvanted with aluminum hydroxide, and a human papillomavirus vaccine adjuvanted with aluminum hydroxyphosphate sulfate, it was shown that vaccines containing a relatively high concentration of aluminum salts (i.e., up to ~1%, w/v, of aluminum hydroxide) can be converted into a dry powder by thin-film freezing followed by removal of the frozen solvent by lyophilization while using low levels of trehalose (i.e., as low as 2% w/v) as an excipient. Importantly, the thin-film freeze-drying process did not cause particle aggregation, nor decreased the immunogenicity of the vaccines. Moreover, repeated freezing-and-thawing of the dry vaccine powder did not cause aggregation. Thin-film freeze-drying is a viable platform technology to produce dry powders of vaccines that contain aluminum salts.

1. Introduction

Some aluminum salts, including aluminum hydroxide and aluminum phosphate, have been used as human vaccine adjuvants for decades. The primary particles of aluminum hydroxide and aluminum phosphate are in the nanometer-scale. However, when dispersed in an aqueous solution, the primary particles aggregate to form larger microparticles of 1–20 μm [1,2]. Thus, a vaccine that is prepared by binding an antigen with an aluminum salt is physically a suspension of aluminum salt particles with antigens adsorbed on them. Many currently licensed and commercially available human vaccines such as diphtheria–tetanus–pertussis, Hepatitis B, and human papillomavirus vaccines contain aluminum salts as adjuvants [3].

A major limiting factor with these vaccines is that they must not be exposed to freezing conditions during transport and storage, and are too fragile to be stable at ambient temperatures. In other words, vaccines that are adjuvanted with aluminum salts must remain stored as a liquid suspension at 2–8 °C from manufacturing to being administered to patients, because inadvertently exposing the suspension to freezing temperatures causes irreversible coagulation that damages the vaccines (e.g., loss in activity and stability) [4]. Vaccines that have been incidentally exposed to freezing conditions before administration to patients must be discarded, causing significant product waste and limited utility. This is significant considering that an estimated 75–100% of the vaccine shipments are actually exposed to freezing temperatures at some points during shipment [5], resulting in costly waste and the loss of nearly half of all global vaccine supplies [6].

There is great interest in addressing this problem, and the strategies to solve it are generally two-fold. The first is to add stabilizing reagents in vaccines to prevent aggregation during freezing. For example, the Program for Appropriate Technology (PATH) and its research collaborators have shown that adding glycerin, polyethylene glycol 300, or

* Corresponding author at: The University of Texas at Austin, College of Pharmacy, Austin, TX 78712, United States.
E-mail address: zhengrong.cui@austin.utexas.edu (Z. Cui).
[1] Contributed equally.

http://dx.doi.org/10.1016/j.jconrel.2015.02.035
0168-3659/

propylene glycol into vaccines that contain aluminum salts prevents vaccine aggregation and preserves vaccine immunogenicity, even after the vaccines are subjected to multiple exposures to −20 °C [4]. Zapata et al. also reported that the adsorption of polymers or surface-active agents, such as hydroxypropyl methylcellulose or polysorbate 80, on aluminum hydroxide prevents aggregation after a freeze–thaw cycle [7]. It is thought that the stabilizing agents produce a large steric repulsive region between particles and hinder particle–particle interactions [7]. However, the addition of the aforementioned excipients into a vaccine may result in a more complex formulation and increase the cost per dose of the vaccine.

Another strategy is to convert aluminum salt-adjuvanted vaccines into a solid form using novel freezing and/or drying techniques. Various methods, such as vacuum-foam drying [8], spray drying [9], spray freeze-drying [10], and spray freezing into liquid [11], have been previously explored to convert protein products into dry powders. Spray freeze-drying has been studied for freeze-drying vaccines that contain aluminum salts [12,13]. For example, using vaccines that were adjuvanted with aluminum hydroxide or aluminum phosphate and contained various excipients (e.g., a mixture of mannitol, glycine, and dextran, or trehalose alone at up to 10 w/v%), Maa and co-workers [10] and Clausi and co-workers [14–16] sprayed atomized liquid vaccine droplets into liquid nitrogen and then successfully lyophilized the frozen particles into dry powder. However, the spray freeze-drying and then reconstitution process either causes particle aggregations or significantly alters the immunogenicity of the vaccines [10,14–16]. Nonetheless, it was concluded that lower aluminum concentration, higher freezing rate, and higher excipient level help minimize adjuvant agglomeration and maximize the immunogenicity of the vaccine [10,15].

Thin-film freezing (TFF) has recently been studied for preparing stable submicron protein particles [17]. In the TFF process, a liquid (e.g., solution) is spread out on a cryogenic substrate to form a thin film in less than one second. The resultant frozen film is then dried by lyophilization. For example, Engstrom et al. produced dried protein powders with a diameter of 300 nm using TFF, and the enzyme activity of the proteins was fully preserved [17]. In the present study, the feasibility of freeze-drying vaccines that are adjuvanted with aluminum salts using TFF was tested. Ovalbumin (OVA) was initially used as a model protein antigen, and it was adjuvanted with aluminum hydroxide or aluminum phosphate and lyophilized after thin-film freezing. The applicability of the thin-film freeze-drying (TFFD) in drying vaccines adjuvanted with aluminum salts was further validated with commercially available veterinary tetanus toxoid vaccine, human hepatitis B vaccine, and human papillomavirus quadrivalent vaccine. The vaccines were evaluated after they were subjected to TFFD and reconstitution to test whether subjecting them to TFFD and reconstitution causes particle aggregation and decreases the immunogenicity of the vaccines. Finally, the dry vaccine powders were also subjected to repeated freezing-and-thawing cycles to test whether freezing conditions cause aggregations.

2. Materials and methods

2.1. Materials

Dried aluminum hydroxide gel (AH gel) and aluminum phosphate were from Spectrum Chemical and Laboratory Products (New Brunswick, NJ). OVA, trehalose, and Laemmli sample buffer were from Sigma-Aldrich (St. Louis, MO). Bio-safe™ Coomassie blue staining solution and Bio-Rad DC™ protein assay reagents were from Bio-Rad Laboratories (Hercules, CA). Alhydrogel® (2%, w/v) was from InvivoGen (San Diego, CA). Tetanus antitoxin concentrated/purified (TT vaccine) was from Colorado Serum Company (Denver, CO). The TT vaccine contains potassium alum (personal communication with Dr. Randall Berrier at Colorado Serum). Potassium alum is also known as potassium aluminum sulfate. Engerix-B, a human hepatitis B vaccine from GlaxoSmithKline, and Gardasil, a human papillomavirus (HPV) quadrivalent vaccine from Merck & Co., Inc., were purchased through the University of Texas at Austin University Health Services. Engerix-B contains aluminum hydroxide (0.5 mg of aluminum per ml). Gardasil contains amorphous aluminum hydroxyphosphate sulfate (0.45 mg/ml of aluminum) as an adjuvant. Mouse Anti-Tetanus Toxoid IgG ELISA kit was from Alpha Diagnostic International (San Antonio, TX). Tetanus toxoid was from List Biologics Laboratory (Campbell, CA). Purified polyclonal horse anti-tetanus serum and guinea pig anti-tetanus IgG were from the National Institute for Biological Standards and Control (Hertfordshire, England).

2.2. Thin-film freeze-drying (TFFD)

Three types of aluminum-containing compounds, dried aluminum hydroxide gel (USP grade) (AH gel to differentiate from commercially prepared Alhydrogel), 2% Alhydrogel®, and aluminum phosphate, were used to adsorb OVA as a model antigen. The OVA-adsorbed aluminum hydroxide vaccine was prepared by mixing an OVA solution with an aluminum hydroxide suspension in phosphate buffered saline (PBS, pH 7.4, 10 mM) to reach an OVA to aluminum weight ratio of 1:10. The vaccine contained 31.4 μg/ml of OVA, 0.09% of aluminum hydroxide, and 0–5% (w/v) of trehalose. The OVA-aluminum phosphate vaccine (31.4 μg/ml of OVA, 0.142% (w/v) of aluminum phosphate, and 2% (w/v) of trehalose) was prepared similarly. When the 2% Alhydrogel® was used, Alhydrogel® (25 ml) was added into a 50 ml tube, followed by the addition of 25 ml of an OVA solution (1 mg/ml) at an OVA to aluminum weight ratio of 1:10, and 1 g of trehalose to obtain a final formulation with 2% (w/v) of trehalose, ~1% (w/v) of Alhydrogel®, and 0.5 mg/ml of OVA. The samples were subjected to TFF and lyophilized as described previously [17,18]. Briefly, the aluminum-containing vaccine suspensions were dropped onto a pre-cooled rotating cryogenic steel surface to form thin films. The thin films were removed by a steel blade. In order to avoid the overlap of two droplets, the speed at which the cryogenic steel surface, on which the vaccine suspension was dropped, was rotating was controlled at 5–7 rpm. The frozen film-like solids were collected in liquid nitrogen and dried using a VirTis Advantage bench top tray lyophilizer (The VirTis Company, Inc. Gardiner, NY). Lyophilization was performed over 72 h at pressures less than 200 mTorr, while the shelf temperature was gradually ramped from −40 °C to 26 °C. After lyophilization, the solid vaccine powder was quickly transferred to a sealed container and stored in a desiccator at room temperature before further use [19].

To dry TT vaccine, trehalose was added into the TT vaccine that was diluted 50-fold in PBS (pH 6.3, 10 mM) to adjust the final concentration of trehalose to 2% (w/v). The vaccine was then subjected to TFFD as mentioned above. To dry Engerix-B, trehalose was added directly into the commercial vaccine (without pre-dilution) to obtain a formulation with 2% (w/v) of trehalose, ~20 μg/ml of HBsAg, and ~500 μg/ml of aluminum, and the vaccine was then subjected to TFFD. In Engerix-B vaccine, each 1-ml adult dose contains 20 μg of HBsAg adsorbed on 0.5 mg of aluminum as aluminum hydroxide. To dry the Gardasil vaccine, 100 μl of the vaccine was diluted to 1 ml of 0.9% (w/v) sodium chloride, and trehalose was added to reach a final concentration of 2% (w/v). The vaccine was then subjected to TFFD. Each 0.5-ml dose of the original Gardasil contains approximately 20 μg of HPV 6 L1 protein, 40 μg of HPV 11 L1 protein, 40 μg of HPV 16 L1 protein, and 20 μg of HPV 18 L1 protein. Each 0.5-ml dose of Gardasil also contains approximately 225 μg of aluminum as amorphous aluminum hydroxyphosphate sulfate.

The morphology of the vaccines in suspension was examined under an Olympus BX60 microscope (Olympus America, Inc., Center Valley, PA). The size of particles and particle size distribution in all samples were determined using a Sympatec Helos laser diffraction instrument (Sympatec GmbH, Germany) equipped with a R3 lens. The moisture in the dried powder was measured using a Karl Fisher Titrator Aquapal III from CSC Scientific Company (Fairfax, VA).