Improving the Stability of Aluminum Salt-Adjuvanted Vaccine to Freezing and Heat by Thin-Film Freeze-Drying

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Purpose

Many currently licensed and commercially available human vaccines such as diphtheria–tetanus–pertussis, Hepatitis B, and human papillomavirus vaccines contain aluminum salts as adjuvants. A major limiting factor with these vaccines is that they must not be exposed to freezing conditions during transport and storage. 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 temperature causes irreversible coagulation that damages the vaccines (e.g., loss in activity and stability). Vaccines that have been incidentally exposed to freezing conditions before administration to patients must be discarded, causing significant product waste and limited utility. However, it is estimated that 75–100% of the vaccine shipments are actually exposed to freezing temperatures at some points during shipment, resulting in costly waste and the loss of nearly half of all global vaccine supplies.

Previously, we reported a novel thin-film freeze-drying (TFFD) method to convert vaccines adjuvanted with insoluble aluminum salts from liquid suspension into dry powder, without affecting particle size and immunogenicity of the reconstituted vaccines. The purpose of this study was to evaluate the immunogenicity of a thin-film freeze-dried, aluminum oxyhydroxide-adjuvanted vaccine powder that was subjected to three cycles of repeated freezing-and-thawing (and then reconstitution) in a mouse model and compared to that of the same freshly prepared vaccine. We also tested the stability of the thin-film freeze-dried, aluminum oxyhydroxide adjuvanted vaccine powder in temperatures as high as 40°C for up to 6 months by measuring the particle size and immunogenicity and compared with the same liquid vaccine.

Methods

The vaccine in present study was prepared by adsorbing ovalbumin (OVA) as a model antigen onto Alhydrogel® (2% w/v, aluminum oxyhydroxide). The vaccine preparation is free of any additional stabilizers or preservatives. The vaccine was converted to dry powder by TFFD using 2% trehalose and was crimp-sealed in a silanized glass vials. For freezing-and-thawing studies, thin-film freeze-dried OVA adsorbed Alhydrogel® powder was placed in -20°C for 8 h and then thawed at 4°C for 16 h for three cycles. After the third cycle, the dry powder was reconstituted to measure its particle size and evaluated its immunogenicity in a mouse model. As a control, OVA-adsorbed Alhydrogel® vaccine in liquid suspension (with 2% of trehalose) was also subjected to 3 cycles of freezing-and-thawing. For heat stability studies, the OVA/Alhydrogel® dry vaccine powder (in desiccator) was stored at room temperature, 30°C, or 40°C for up to 6 months. At various time points, the particle size and immunogenicity of the vaccine were evaluated after reconstitution. As a control, the stability of the OVA/Alhydrogel® vaccine in liquid suspension was evaluated.

Results

Serum anti-OVA IgG levels in the mice immunized with OVA/Alhydrogel® reconstituted from the dry powder that was subjected to three cycles of freezing-and-thawing are not different from that in mice that were immunized with freshly prepared OVA/Alhydrogel® liquid vaccine. On the other hand, serum anti-OVA IgG levels in the mice immunized with OVA/Alhydrogel® liquid vaccine that was subjected to three cycles of freezing-and-thawing were significantly lower than that in mice that were immunized with freshly prepared OVA/Alhydrogel® liquid vaccine (p < 0.05). Also, subjecting the OVA/Alhydrogel® vaccine dry powder to repeated freezing-and-thawing did not cause significant particle aggregation, whereas significant aggregations were observed after the OVA/Alhydrogel® liquid vaccine was subjected to repeated freezing-and-thawing.

For heat stability studies, no significant particle aggregation was observed after the OVA/Alhydrogel® dry vaccine powder was stored for 3 months at the various temperatures tested. While the liquid vaccine started showing significant aggregations after only one month of storage at different temperatures. The OVA/Alhydrogel® vaccine dry powder after storing at various temperatures for 3 months was as immunogenic as freshly prepared OVA/Alhydrogel® vaccine, whereas the OVA/Alhydrogel® liquid vaccine stored at room temperature for 3 months became significantly less immunogenic as compared freshly prepared OVA/Alhydrogel® liquid vaccine.

Conclusion

Converting an aluminum salt-adjuvanted vaccine from liquid suspension to dry powder using TFFD renders the vaccine stable to freezing and heat.