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Incompatibility of lyophilized inactivated polio vaccine with liquid pentavalent whole-cell-pertussis-containing vaccine

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ABSTRACT

A hexavalent vaccine containing diphtheria toxoid, tetanus toxoid, whole cell pertussis, *Haemophilius influenza* type B, hepatitis B and inactivated polio vaccine (IPV) may: (i) increase the efficiency of vaccination campaigns, (ii) reduce the number of injections thereby reducing needlestick injuries, and (iii) ensure better protection against pertussis as compared to vaccines containing acellular pertussis antigens. An approach to obtain a hexavalent vaccine might be reconstituting lyophilized polio vaccine (IPV-LYO) with liquid pentavalent vaccine just before intramuscular delivery. The potential limitations of this approach were investigated including thermostability of IPV as measured by D-antigen ELISA and rat potency, the compatibility of fluid and lyophilized IPV in combination with thimerosal and thimerosal containing hexavalent vaccine.

The rat potency of polio type 3 in IPV-LYO was 2 to 3-fold lower than standardized on the D-antigen content, suggesting an alteration of the polio type 3 D-antigen particle by lyophilization. Type 1 and 2 had unaffected antigenicity/immunogenicity ratios. Alteration of type 3 D-antigen could be detected by showing reduced thermostability at 45 °C compared to type 3 in non-lyophilized liquid controls.

Reconstituting IPV-LYO in the presence of thimerosal (TM) resulted in a fast temperature dependent loss of polio type 1-3 D-antigen. The presence of 0.005% TM reduced the D-antigen content by ~20% (polio type 2/3) and ~60% (polio type 1) in 6 h at 25 °C, which are WHO open vial policy conditions. At 37 °C, D-antigen was diminished even faster, suggesting that very fast, i.e., immediately after preparation, intramuscular delivery of the conceived hexavalent vaccine would not be a feasible option. Use of the TM-scavenger, L-cysteine, to bind TM (or mercury containing TM degradation products), resulted in a hexavalent vaccine mixture in which polio D-antigen was more stable.

1. Introduction

Combination vaccines are very successful, especially for delivery in children. The inclusion of multiple vaccine antigens in a single formulation reduces the number of injections, facilitates inclusion of new vaccines and increases coverage of routine pediatric immunization programs. For example, the use of pentavalent vaccine combining diphtheria-tetanus-pertussis (DTP), *Haemophilus influenzae* type B (Hib) and hepatitis B (HBV) antigens has raised the coverage of Hib and hepatitis B in the poorest developing (Gavi-supported) countries [1].

One of the challenges for an IPV-containing hexavalent vaccine is the presence of the preservative thimerosal (TM). TM negatively affects the antigenicity and immunogenicity of IPV [2] and is used in the production process of whole cell pertussis (wP) vaccine as an inactivating agent as well as a preservative [3]. Hence, pentavalent vaccine contains trace amounts of TM (⩽0.01% (w/v)).

Currently, the globally marketed IPV-containing hexavalent pediatric combination vaccines (Infanrix Hexa® (GSK) and Hexaxim® (Sanofi Pasteur)) contain an acellular pertussis (aP) component, which is devoid of TM. The use of wP in hexavalent vaccines intended for developing countries is important because of the lower costs and emerging doubts about the long-term effectiveness of aP vaccines. Unfortunately, no hexavalent combinations with wP (without TM) are licensed or in late-stage development [1].

The aim of this study is to investigate whether IPV-LYO, as previously developed [4], could be used in combination with a wP-containing pentavalent vaccine to generate a concept hexavalent

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vaccine, for example, for use in developing countries. By reconstituting IPV-LYO with pentavalent vaccine no substantial change in total volume is anticipated, likely the same injection volume for IM-injection may be used.

This study addresses the (thermo)stability of IPV-LYO with respect to both D-antigenicity and immunogenicity (rat potency) and shows D-antigenicity data on IPV-LYO reconstituted with a pentavalent vaccine (DTwP-Hib-HBV).

2. Materials and methods

2.1. Materials

The IPV used in this study is a ten times concentrated trivalent bulk containing the inactivated Mahoney (type 1), MEF (type 2) and Saukett (type 3) strains at a nominal D-antigen content (expressed in D-units, DU) of 400-80-320 DU/mL (for types 1, 2 and 3, respectively) and produced under cGMP conditions according to a routine production process [5]. The pentavalent vaccine, Diphtheria, Tetanus, (whole cell) Pertussis, Hepatitis B and *Heamophilus influenza* type b Conjugate Vaccine Adsorbed, was a gift from Serum Institute of India (SII).

D-sorbitol, magnesium chloride hexahydrate ($MgCl_2{\cdot}6H_20$) and monosodium glutamate monohydrate were from Sigma-Aldrich (St. Louis, MO). Citric acid (Sigma-Aldrich, St. Louis, MO) and disodium hydrogen phosphate (Fluka, Buchs, Switzerland) were used to prepare McIlvaine buffer. Thimerosal (TM) and L-cysteine were from Sigma-Aldrich (St. Louis, MO). All excipients used were of reagent quality or of a higher grade.

2.2. Methods

2.2.1. Formulating IPV

Unless indicated otherwise, the trivalent IPV bulk material was dialyzed against 10 mM McIlvaine buffer (pH 7.0) using a low-binding regenerated cellulose membrane dialysis cassette (M_w cut-off = 10 kDa). The dialyzed IPV was diluted 1:1 with formulation buffer containing: D-sorbitol (20% w/v), $MgCl_2{\cdot}6H_20$ (17% w/v), and monosodium glutamate monohydrate (17% w/v) in McIlvaine buffer (10 mM, pH 7.0). This formulated IPV was used for the preparation of IPV-LYO. Liquid IPV was prepared by 1:1 dilution of (not dialyzed) trivalent IPV with ultrapure water. This material was used as a control in experiments.

2.2.2. Lyophilization

Injection vials (3 mL, Aluglas BV, Uithoorn, The Netherlands) were filled with 0.2 mL of the formulated IPV and half-stoppered with pre-dried (overnight at 105 °C) 13 mm lyophilization stoppers (PH21/50 from Aluglas BV, Uithoorn, The Netherlands). Vials were loaded on precooled shelves (−50 °C) and the solidified material was subsequently lyophilized. Primary drying was done at 0.045 mbar and −45 °C for 26 h. Secondary drying was done at a pressure of 0.01 mbar and shelf temperature that increased from −45 °C to 25 °C in 13.3 h. Thereafter, both shelf temperature (25 °C) and pressure (0.01 mbar) were kept constant for 24 h. After lyophilization, vials were closed under vacuum, sealed with alucaps and stored for stability testing.

2.2.3. Stability testing

For stability studies, liquid IPV (0.2 mL in stoppered and capped 3 mL injection vials) and IPV-LYO (in stoppered and capped 3 mL vials) were incubated at 2-8, 25, 37, and 45 °C. After various periods of time, vials were taken for analysis.

2.2.4. Effect of thimerosal

The effect of thimerosal (TM) on liquid IPV was studied by diluting trivalent IPV 10-fold with a solution of TM in ultrapure water. IPV-LYO was reconstituted either with ultrapure water (0.5 mL), 0.5 mL TM solution, or 0.5 mL pentavalent SII-vaccine containing 0.005% (w/v) TM. Final TM concentrations were 0.005 and 0.01% (w/v).

The possible neutralizing effect of L-cysteine on TM was investigated by pre-incubating pentavalent vaccine for one hour with 0.05% (w/v) L-cysteine or ultrapure water as negative control. Subsequently, IPV-LYO was reconstituted with the pre-incubated pentavalent vaccines or with ultrapure water as a control. D-antigen recoveries were determined directly after mixing or after subsequent storage at 37 °C for 24 h.

2.2.5. Analysis

2.2.5.1. D-antigen ELISA. The D-antigen ELISA was performed as described elsewhere [6]. Microtiter plates were coated with serotype-specific bovine anti-polio serum (Bilthoven Biologicals, Bilthoven, The Netherlands). After washing, dilutions of IPV (reconstituted IPV-LYO or liquid) were added. After an incubation period of 30 min at 37 °C under gentle shaking, plates were washed. Subsequently, a mixture of serotype-specific anti-poliovirus monoclonal antibody (3-4E4, 3-14-4 and 1-12-9 for type 1, 2 and 3, respectively) and HRP-labeled anti-mouse IgG (GE Healthcare, Buckinghamshire, UK) was added and plates were incubated for 30 min. at 37 °C while shaking. Subsequently, plates were washed followed by addition of ELISA HighLight signal reagent (ZomerBloemen BV, Zeist, The Netherlands). Chemiluminescence was measured for 10–15 min by using a luminometer (Berthold Centro LB960). The signal at maximum intensity was used to calculate the D-antigen content relative to the reference standard.

2.2.5.2. Biosensor analysis. Antigenicity was also measured using a Biacore T200 (GE Healthcare, Hoevelaken, The Netherlands), equipped with an anti-polio biosensor as described elsewhere [7]. Goat anti-mouse IgG Fc-specific (Thermo Fisher Scientific Inc, Waltham, MA), antibodies were covalently immobilized on the dextran layer of a CM3 sensorchip (GE Healthcare, Hoevelaken, The Netherlands) by primary amine coupling, following the manufacturers recommendations (GE Healthcare, Hoevelaken, The Netherlands). Serotype-specific monoclonal antibodies (3-4E4 (antigenic site 1, type 1), 3-14-4 (antigenic site 1, type 2), Hyb300-06 (antigenic site 1, type 3) and 1-12-9 (antigenic site 2/3/4, type 3) were bound to the sensor, followed by IPV. The sensor chip was regenerated with 10 mM glycine-HCl (pH 1.5). Assay data were analyzed by four-parameter curve fitting using the Biacore T200 evaluation software. Antigenicity was calculated relative to the international reference PU91-01.

2.2.5.3. Rat potency. Immunogenicity of IPV-LYO was measured in the rat potency test performed as described earlier [7] with the exception that the highest dilution of the vaccine was not included. Animal experiments were conducted in accordance with the guidelines provided by the Dutch Animal Protection Act, and were approved by the Committee for Animal Experimentation of Intravacc. RIVM-TOX rats were immunized with four threefold dilutions of reconstituted IPV-LYO, the liquid IPV control, and the reference vaccine (PU91-01). After three weeks, sera were collected and neutralizing antibodies against all three poliovirus types were measured separately by inoculating Vero cells with 100 $TCID_{50}$ of the wild-type strains (Mahoney, MEF-1 and Saukett) as described previously [8]. Two-fold serial serum dilutions were made and serum/virus mixtures were incubated for three hours at 36 °C and 5% CO_2 followed by overnight incubation at 5 °C. Subsequently, Vero cells were added and after 7 days of incubation at 36 °C and 5% CO_2 the

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