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Intranasal and sublingual delivery of inactivated polio vaccine

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ABSTRACT

Polio is on the brink of eradication. Improved inactivated polio vaccines (IPV) are needed towards complete eradication and for the use in the period thereafter. Vaccination via mucosal surfaces has important potential advantages over intramuscular injection using conventional needle and syringe, the currently used delivery method for IPV. One of them is the ability to induce both serum and mucosal immune responses: the latter may provide protection at the port of virus entry.

The current study evaluated the possibilities of polio vaccination via mucosal surfaces using IPV based on attenuated Sabin strains. Mice received three immunizations with trivalent sIPV via intramuscular injection, or via the intranasal or sublingual route. The need of an adjuvant for the mucosal routes was investigated as well, by testing sIPV in combination with the mucosal adjuvant cholera toxin.

Both intranasal and sublingual sIPV immunization induced systemic polio-specific serum IgG in mice that were functional as measured by poliovirus neutralization. Intranasal administration of sIPV plus adjuvant induced significant higher systemic poliovirus type 3 neutralizing antibody titers than sIPV delivered via the intramuscular route. Moreover, mucosal sIPV delivery elicited polio-specific IgA titers at different mucosal sites (IgA in saliva, fecal extracts and intestinal tissue) and IgA-producing B-cells in the spleen, where conventional intramuscular vaccination was unable to do so. However, it is likely that a mucosal adjuvant is required for sublingual vaccination. Further research on polio vaccination via sublingual mucosal route should include the search for safe and effective adjuvants, and the development of novel oral dosage forms that improve antigen uptake by oral mucosa, thereby increasing vaccine immunogenicity. This study indicates that both the intranasal and sublingual routes might be valuable approaches for use in routine vaccination or outbreak control in the period after complete OPV cessation and post-polio eradication.

1. Introduction

During the past decades, the global incidence of paralytic poliomyelitis has decreased by more than 99% since 1988. Type 2 wild poliovirus was eradicated in 1999 and the last reported case of type 3 wild poliovirus was from 2012. Since 2015, cases of type 1 wild poliovirus were only detected in the remaining endemic countries (i.e., Pakistan, Afghanistan and Nigeria) [1]. It is expected that wild poliovirus will be eradicated within a few years. However, to accomplish a polio-free world, eradication efforts should focus on both wild polioviruses as well as vaccine-derived viruses. Therefore, the endgame strategy of the Global Polio Eradication Initiative (GPEI) includes a phased withdrawal of the live-attenuated oral polio vaccine (OPV), the source of vaccine-derived viruses, and the worldwide inclusion of the inactivated polio vaccine (IPV) into all routine immunization programs [2]. The GPEI is pursuing several priority approaches for the development of a new generation of IPV [3]. To this extent, Intravacc has developed a new polio vaccine based on Sabin polio viruses, Sabin IPV (sIPV), that is being transferred to local vaccine manufacturers to support post-eradication goals in terms of biosafety and IPV availability [4–7].

A new generation of sIPV should not only be affordable and safe to produce, but preferably should also induce mucosal immunity, remain stable, and be easy to administer. This is important with regard to stockpiling and outbreak management in the period after cessation of OPV and after eradication. Several alternative polio vaccine delivery strategies are in development, with a focus on dermal delivery of polio vaccines [8]. Vaccination via mucosal sites has the benefits of needle free vaccine delivery. Moreover, mucosal immunization is able to elicit strong mucosal immunity, even at distant effector sites. As we know from OPV, polio-specific mucosal

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immunity in the gut is a powerful mechanism for protection and interruption of polio transmission [9].

The current study evaluated the potential of different mucosal routes, i.e., intranasal and sublingual, in mice. It was investigated whether intranasal or sublingual vaccination with sIPV is able to elicit functional systemic immunity (serum) as well as local immune responses at different mucosal sites.

2. Materials and methods

2.1. Vaccine

Monovalent Sabin IPV bulk material used in this study was produced as described previously [10]. For the preparation of trivalent sIPV, monovalent type 1, type 2 and type 3 were mixed and diluted in M199 medium (Bilthoven Biologicals, The Netherlands) to a nominal concentration of 1000–1600–3200 D-antigen units (DU) per mL for type 1, type 2 and type 3, respectively. Cholera toxin from *Vibrio Cholerae* was purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Immunization study

The animal experiment was performed according to the guidelines provided by the Dutch Animal Protection Act, and was approved by the Committee of Animal Experimentation (DEC) of the National Institute of Public Health and Environment (RIVM). Balb/cOlaHsd mice (8–10 weeks old from Envigo, The Netherlands) were anesthetized with ketamine-xylazine, and received a single human dose (based on previous clinical studies [6,11]) trivalent sIPV (10–16–32 DU/dose) via the intramuscular (IM, injection of 50 μL in hind limb), intranasal (IN, pipetting 10 μL in the nose) or sublingual (SL, pipetting 10 μL under the tongue) route at day 0, 7 and 28. Adjuvanted groups received 5 μg/dose cholera toxin. Upon SL immunization, mice were maintained in upright position to minimize the risk of swallowing. Blood samples were taken at day 0 (prior to immunization) and day 14 (after second immunization). At day 35, anesthetized animals received an intraperitoneal injection of 0.1 mL of 0.05 M pilocarpine (Sigma-Aldrich, St. Louis, MO) in PBS to induce saliva production. Saliva was collected and, subsequently, animals were sacrificed by bleeding. Post-mortem, fecal samples were isolated from the large intestine, weighted and stored at −80 °C until analysis. Spleens were placed in RPMI-1640 medium (Gibco, Invitrogen) supplemented with 5% fetal bovine serum and placed on ice for the B-cell ELISPOT. Small intestines were harvested and placed in 3 mL PBS containing 50 mM EDTA (Gibco, Invitrogen) and protease inhibitors (Complete, Mini, EDTA free, Roche Applied Sciences). Small intestines were extensively vortexed and centrifuged for 15 min at 300*g* (4 °C). Supernatants, mentioned further as intestinal wash, were collected and stored at −80 °C until analysis (IgA ELISA). Subsequently, small intestines were cut into small pieces, transferred to cryotubes, and 2 μL PBS containing 2% saponin (Sigma Aldrich, St. Louis, MO) and protease inhibitors was added per mg intestinal sample. After a fast freezing step, samples were centrifuged for 20 min at 4600 rpm and supernatants were filtered through 0.22 μm filters (Merck Millipore, Darmstadt, Germany). Intestinal tissue samples were stored at −80 °C until further analysis. The presence of both excreted (intestinal washes) and intracellular (intestinal tissue samples) polio-specific IgA in small intestine was assessed by ELISA.

2.3. IgG and IgA ELISA

Enzyme linked immunosorbent assays (ELISA) were performed to determine polio-specific antibody titers in sera, saliva, feces, intestinal washes and intestinal tissue samples. Fecal extracts were prepared by adding fecal extract buffer, PBS containing 10% normal goat serum (Sigma Aldrich, St. Louis, MO) and protease inhibitors, to the fecal pellets (0.2 g/mL). Fecal extracts were extensively vortexed and, subsequently, centrifuged for 15 min at 13,000 g. Supernatants were filtered through 0.22 μm filters and immediately tested. For the ELISA, polystyrene 96 wells microtiter plates (Greiner Bio-One, Alphen a/d Rijn, The Netherlands) were coated overnight at 4 °C with bovine anti-poliovirus serum (Bilthoven Biologicals, Bilthoven, The Netherlands) in PBS (Gibco from Invitrogen, Paisley, UK). After washing coated plates with 0.05% (v/v) Tween 80 (Merck, Darmstadt, Germany) in tap water, trivalent inactivated polio vaccine diluted in assay buffer, PBS containing 0.5% (w/v) Protifar (Nutricia, Zoetermeer, The Netherlands) and 0.05% (v/v) Tween 80 (Merck, Darmstadt, Germany), was added and incubated for 2 h at 37 °C. Subsequently, plates were washed and threefold sample dilutions in assay buffer were added and incubated for another 2 h at 37 °C. After washing, plates were incubated with horse-radish peroxidase (HRP)-conjugated goat-anti-mouse IgG or HRP-conjugated goat-anti-mouse IgA (Southern Biotech, Birmingham, AL). After 1 h incubation at 37 °C, plates were washed and TMB substrate solution, containing 1.1 M sodium acetate (Bilthoven Biologicals, Bilthoven, The Netherlands), 100 mg/mL 3,3′,5,5′-tetramethylbenzidine (Sigma-Aldrich, St. Louis, MO), and 0.006% (v/v) hydrogen peroxide (Merck, Darmstadt, Germany), was added to each well. After 10–15 min., the reaction was stopped with 2 M sulfuric acid (Bilthoven Biologicals, Bilthoven, The Netherlands) and absorbance was measured at 450 nm by using a Biotek L808 plate reader. For the CT-specific ELISA, plates were coated with 1 μg/mL CT and blocked with 1% Protifar in PBS. ELISA was further performed as described above. Endpoint titers were determined by 4-parameter analysis using the Gen5™ 2.0 Data Analysis software (BioTek Instruments, Inc., Winooski, VT) and defined as the reciprocal of the serum dilution producing a signal identical to that of negative control samples at the same dilution plus three times the standard deviation.

2.4. Virus neutralization (VN) assay

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 [12,13]. Twofold 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 plates were stained and fixed with crystal violet and results were read macroscopically. Virus neutralizing (VN) titers were expressed as the last serum dilution that has an intact monolayer (no signs of cytopathogenic effect).

2.5. B-cell ELISPOT

MultiScreen-HTS IP 96 wells filter plates (Merck Millipore, Darmstadt, Germany) were wet by adding 35% ethanol, immediately washed twice with PBS and, subsequently, coated overnight with 5 μg/mL monovalent IPV type 1, 2 or 3. As a positive control, wells were coated with a mixture of 7 μg/mL purified goat-anti-mouse kappa and 7 μg/mL purified goat-anti-mouse lambda (Southern Biotech). As a negative control, wells were left uncoated (PBS). After washing with PBS, plates were blocked with RPMI-1640 medium (Gibco, Invitrogen) with 2% Protifar (Nutricia, Zoetermeer, The Netherlands) for 1 hour at room temperature. Spleens were homogenized using a 70-μm cell strainer (BD Falcon, BD Biosciences) and cells were collected in RPMI-1640 supplemented with 10% fetal bovine serum and antibiotics (Penicillin-St

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