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Characterization and standardization of Sabin based inactivated polio vaccine: Proposal for a new antigen unit for inactivated polio vaccines

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ABSTRACT

GMP-batches of Sabin-IPV were characterized for their antigenic and immunogenic properties. Antigenic fingerprints of Sabin-IPV reveal that the D-antigen unit is not a fixed amount of antigen but depends on antibody and assay type. Instead of the D-antigen unit we propose standardization of IPV based on a combination of protein amount for dose and D-antigenicity for quality of the vaccine. Although Sabin-IPV type 2 is less immunogenic than regular wild type IPV type 2, the immunogenicity (virus neutralizing titers) per microgram antigen for Sabin-IPV type 2 is in the same order as for wild type serotypes 1 and 3. The latter observations are in line with data from human trials. This suggests that a higher dose of Sabin-IPV type 2 to compensate for the lower rat immunogenicity may not be necessary.

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1. Introduction

Thanks to the Sabin oral poliovirus vaccine (OPV), the wild-type inactivated poliovirus vaccine (wt IPV) and the successful WHO campaign for Global Eradication of Polio [1,2] eradication of wild-type polio virus is at hand. One major disadvantage of attenuated OPV is that it can cause vaccine-associated paralytic poliomyelitis or outbreaks of vaccine-derived poliovirus [3]. If because of that OPV use is discontinued, the only vaccine available to induce and maintain immunity against polio will be IPV. To prepare for the post-eradication period and meet the demands for IPV, the production capacity of wt IPV would need to increase. However a major drawback of producing wt IPV is that the strains used require stringent containment measures, which restricts production to industrialized countries [4]. Therefore the development of Sabin IPV that can be produced economically on an large scale in developing countries may constitute an attractive option.

WHO has requested NVI to develop a safe and effective candidate Sabin IPV. Different studies have reported the poor immunogenicity of Sabin type 2 and the lack of comparability between wt IPV and Sabin IPV with regard to antigenicity [5–8]. IPV antigenicity is expressed in D-antigen units. A complicating matter in the comparison of antigenic and immunogenic properties is the fact that the D-antigen is not well defined. Manufacturers and Official Medicines Control Laboratories use their own antibodies and procedures. Collaborative studies have shown that this often causes problems when IPV-samples have to be quantified. This is the case for both antigenicity [9] as well as in immunoassays [10].

In this paper we have extended the study on antigenic and immunogenic properties of Sabin IPV with a panel of monoclonal antibodies (mabs) and two different methods to come to a precise antigenic fingerprint of Sabin IPV. By quantification of protein and virus and measuring potency, we were able to compare antigenic and immunogenic profiles of Sabin IPV and wt IPV. Based on the results of the characterization and standardization study we propose a better defined unit for inactivated poliovirus vaccines.

2. Materials and methods

2.1. Vaccine preparation

wt IPV and International Reference (Pu91-01) [11] were produced under cGMP conditions according to a routine production process [12,13]. Sabin IPV was produced under cGMP according to a slightly modified wt IPV production process. Vero cells were cultivated on micro carriers (Cytodex 1, GE Healthcare) suspended in fermentors, followed by infection with wild strains (Mahoney, MEF-1 or Saukett) or Sabin (LSc 2ab KP_2; $P7_{12}$ Ch2ab-KP_2 or Pfizer 457-III) strains. Virus was purified by filtration (clarification followed by ultra filtration, both Millipore), gel permeation

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chromatography on Sepharose CL-6B (GE Healthcare) and ion exchange chromatography on either DEAE Sepharose Fast Flow or DEAE Sephadex A-50 (both GE Healthcare) in a phosphate buffer. To the processed virus, M199 and glycine (final concentration 5 g/L) was added and the fluid was filtered through a 0.22 μm pore size filter (Millipak-200, Millipore) prior to inactivation. Inactivation was performed using 0.025% formalin during 13 days at 37 °C according World Health Organization (WHO) requirements [14].

For each serotype two batches monovalent pools were produced (Sabin IPV A and B) and from that two trivalent final bulks were composed. One other batch per serotype Sabin IPV was produced on lab-scale (Sabin IPV C), directly from the working seedlots. Sabin final bulk was formulated as 10-16-32 DU/single human dose (shd) (0.5 ml) for types 1, 2 and 3, respectively. The wt IPV reference (Pu91-01; 430-95-285 DU/ml) was formulated as 40-8-32 DU/shd.

2.2. Antibodies

D-specific anti-type 1 (17C5M1, 3-4E4), anti-type 2 (10E8D5, 3-14-4) and anti-type 3 (4-8-7 and 1-12-9) mabs were obtained after immunization with IPV from Mahoney, IPV from MEF (10E8D5) or trypsin treated MEF (3-14-4) and trypsin treated Saukett virus (4-8-7 and 1-12-9), respectively [15]. Mabs 234, 237, Sabin specific mab 423 (all three anti-type 1), 1037, 1050, 1103 (all three anti-type 2) and 204 (anti-type 3) were prepared and characterized at NIBSC. Mabs Hyb295-15/17 (anti-type 1), Hyb294-02/06 (anti-type 2) and Hyb300-05/06 (anti-type 3) were commercially available (BioPorto).

2.3. ELISA

Polystyrene 96-well plates were coated overnight at room temperature with bovine anti-polio serum and blocked with 1% BSA (Sigma–Aldrich) for 30 min at 37 °C. The plates were washed with tap-water containing 0.05% Tween 80. A series of eight twofold dilutions of vaccine in 0.01 M PBS containing 0.05% Tween 80 was added to each plate and incubated at 37 °C for 2 h (note: QC-ELISA: incubation 37 °C for 2 h and overnight at 4 °C). The unbound antigen was then removed and the plates were washed as above. Type-specific mabs were added and the plates were incubated at 37 °C for 2 h. After washing the plate, HRPO-conjugated goat-anti-mouse IgG (Southern Biotech) was added to each well, followed by incubation at 37 °C for 2 h. Plates were then washed and tetramethylbenzidine (TMB) substrate (Sigma–Aldrich) was added. After 10 min the reaction was stopped by addition of 0.2 M H_2SO_4 and absorbance at 450 nm was measured. Assay data were analysed by four-parameter logistic curve fitting. D-antigen units were calculated relative to the reference preparation PU91-01.

2.4. Biosensor analysis

Antigenicity was also measured in a Biacore T100 (GE Healthcare), equipped with an anti-polio biosensor. Goat anti-mouse IgG F_c-specific (Thermo Scientific Inc.), antibodies were covalently immobilized on the dextran layer of a CM3 sensorchip (GE Healthcare) by primary amine coupling, following the manufacturers recommendations (GE Healthcare). Mabs were bound to the sensor, followed by IPV. The sensor chip was regenerated with 10 mM glycine-HCl, pH 1.5. Assay data were analysed by a four-parameter logistic curve fitting using the Biacore T100 evaluation software. Antigenicity was calculated relative to the international reference PU91-01.

Particle concentrations were measured in a Biacore T100 by the calibration free concentration analysis (CFCA). Specific mabs were immobilized to a CM3-sensorchip through a capture approach. Monovalent vaccines were diluted to 1–2 μg/ml protein and injected during 36 s at two different flow rates (5 and 100 μl/min). The observed binding data were fitted to a mass transport-limited 1:1 interaction model with a known value for the mass transport coefficient (derived from the diffusion coefficient of polio virus [16]) and an unknown variable for the analyte concentration.

2.5. Potency testing

Immunogenicity of vaccines was measured in the rat potency test [9,17]. RIVM-TOX rats (10 per dilution) were immunized once with 5 threefold dilutions of the vaccine and the reference vaccine (Pu91-01). After three weeks sera were collected. Neutralizing antibodies against all three poliovirus types (Mahoney, MEF-1 and Saukett) were measured separately using 100 $TCID_{50}$ of the wild-type strains as challenge viruses and Vero cells as indicator cells. Serum dilutions-virus incubation was 3–5 h at 36 °C and overnight at 4 °C [18]. Results were read after 7 days of incubation at 36 °C and the virus neutralizing titers were expressed as a score, which is the number of the last serum dilution with an intact monolayer (no signs of cytopathologic effect (CPE)). Immunogenicity was expressed in two ways: (1) as the relative potency (to the reference vaccine) using the parallel-line model; (2) as the average virus neutralizing antibody titer of 10 rats at the highest vaccine dose.

2.6. Antigen concentrations

Protein concentrations were determined by the method of Bradford. The amount of virus in the monovalent pools was calculated from the UV absorbance at 260 nm of the purified monovalent bulk intermediate after IEX. The extinction of a polio virus solution of 1% (w/v) is 74 AU at 260 nm [19,20].

3. Results

3.1. Immunogenicity in rats

The immunogenicity of Sabin IPV batches A and B are compared with the wt IPV international reference (Fig. 1A–C). The immunogenicity of Sabin type 1 per DU is higher than the immunogenicity of the wt IPV reference (Pu91-01). The immunogenicity of Sabin IPV type 2 is much lower as compared to Pu91-01. Both Sabin and Pu91-01 type 3 have comparable immunogenicity. This confirms earlier findings [5–7,21].

The relative potencies of two trivalent final lots Sabin IPV, formulated with the Sabin IPV bulk products A and B are shown in Table 1.

The neutralizing antibody titer of Sabin IPV type 2 is on average 8 times higher than the neutralizing antibody titer of Sabin IPV type 1 and comparable ($p = 0.08$; ANOVA, $p < 0.05$) with the neutralizing antibody titer of Sabin IPV type 3. The neutralizing antibody titer of wt IPV type 2 is even 32 times higher than that of wt IPV type 1 and four times higher than the neutralizing antibody titer of wt IPV type 3.

The two trivalent Sabin IPVs will be tested in a pre-clinical and a phase 1 clinical study. For a proper formulation of the vaccines the neutralizing antibody titer in rats should be equal or higher than the antibody titer of the international reference.

Fig. 2A and B shows that for both Sabin IPV types 1 and 2 at doses between 10 and 20 DU the neutralizing antibody titers are comparable with the antibody titers of the reference. Sabin type 3 has comparable titers at a dose of 30–40 DU (Fig. 2C).

3.2. Antigenic fingerprint

In a previous paper we stated that the D-antigen unit represents two different entities (antigenic content and immunogenic

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