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Antibody responses of *Macaca fascicularis* against a new inactivated polio vaccine derived from Sabin strains (sIPV) in DTaP-sIPV vaccine

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ABSTRACT

Antibody responses of *Macaca fascicularis* against a new tetravalent vaccine composed of diphtheria toxoid, tetanus toxoid, acellular pertussis antigens, and inactivated poliovirus derived from Sabin strains (sIPV) was investigated to predict an optimal dose of sIPV in a new tetravalent vaccine (DTaP-sIPV) prior to conducting a dose-defined clinical study. Monkeys were inoculated with DTaP-sIPVs containing three different antigen units of sIPVs: Vaccine A (types 1:2:3 = 3:100:100 DU), Vaccine B (types 1:2:3 = 1.5:50:50 DU), and Vaccine C (types 1:2:3 = 0.75:25:25 DU). There was no difference in the average titers of neutralizing antibody against the attenuated or virulent polioviruses between Vaccines A and B. The average neutralizing antibody titers of Vaccine C tended to be lower than those of Vaccines A and B. The sIPV antigens did not affect the anti-diphtheria or anti-tetanus antibody titers of DTaP-sIPV. Furthermore, the average neutralizing antibody titers of Vaccine A against the attenuated and virulent polioviruses were comparable between *M. fascicularis* and humans. These results suggest that *M. fascicularis* may be a useful animal model for predicting the antibody responses to sIPVs in humans, and that it may be likely to reduce the amount of sIPVs contained in DTaP-sIPVs, even for humans.

1. Introduction

Poliomyelitis is a neurological disorder caused by poliovirus and clinically manifests as acute flaccid paralysis [1,2]. As no antiviral drug against polioviruses is commercially available yet, vaccines play an important role in preventing polio and controlling epidemics. There are currently two kinds of vaccine: oral polio vaccines (OPVs) and inactivated polio vaccines (IPVs) [2–4]. Although OPVs are safe and effective, polio associated with the vaccine or vaccine-derived polioviruses derived from OPVs have been highlighted as a significant problem in areas where polio has been eradicated. Therefore, many countries have been shifting from OPVs to IPVs [5,6]. There are two kinds of IPV: conventional IPVs produced using virulent polioviruses (cIPVs), and novel IPVs produced using attenuated Sabin strains (sIPVs).

At the final stage of polio eradication, production of cIPVs will require a higher biosafety level than the one in current use [2]. To make production of IPVs safer, the WHO has encouraged new manufacturers to consider the production of sIPVs [7].

The Japan Poliomyelitis Research Institute (JPRI), the Netherlands Vaccine Institute (NVI), and other Institutes have been developing sIPVs [8]. Some reports suggest that the immunogenicity of type 1 sIPV is higher than that of cIPVs. On the other hand, the immunogenicity of type 2 sIPV is lower than that of cIPVs. Type 3 sIPV and cIPVs are comparable with respect to immunogenicity [9,10]. Therefore, the number of D-antigen units (DU) of each type of sIPV is needed to adjust the content of antigen in the vaccine so that it will induce neutralizing antibody titers equal to those of cIPVs. It was then confirmed that 3 DU, 100 DU, and 100 DU of types 1, 2 and 3 sIPVs, respectively, had the same immunogenicity in rats as cIPVs [11].

Based on this information, prior to conducting a dose-defined clinical study, we tried to predict an optimal dose of sIPV in DTaP-sIPV using *Macaca fascicularis* as a model.

2. Materials and methods

2.1. Vaccine

Three kinds of DTaP-sIPVs containing different D-antigen units of sIPV were prepared using sIPVs produced by JPRI together with DTaP produced by Kaketsuken. The D-antigen units of sIPVs for types 1, 2, and 3 in DTaP-sIPV were adjusted to 3 DU, 100 DU, and 100 DU for Vaccine A; 1.5 DU, 50 DU, and 50 DU for Vaccine B; and 0.75 DU, 25 DU, and 25 DU for Vaccine C (Table 1). The amounts of

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Table 1
Composition of the active ingredients in the DTaP-sIPV vaccines.

Active ingredient	Antigen amounts[a] of DTaP-sIPV vaccines		
	Vaccine A	Vaccine B	Vaccine C
sIPV (type 1)	3.0 DU	1.5 DU	0.75 DU
sIPV (type 2)	100 DU	50 DU	25 DU
sIPV (type 3)	100 DU	50 DU	25 DU
Diphtheria toxoid	≤16.7 Lf	≤16.7 Lf	≤16.7 Lf
Tetanus toxoid	≤6.7 Lf	≤6.7 Lf	≤6.7 Lf
Pertussis component	≥4 U	≥4 U	≥4 U

[a] Antigen amounts per dose (0.5 mL).

DTaP and aluminum adjuvant components contained in Vaccines A, B, and C were the same as the amounts contained in the licensed DPT "KAKETSUKEN" Syringe. The acellular pertussis antigens in the vaccine consisted of pertussis toxin (PT) and filamentous hemagglutinin (FHA) treated with formalin.

2.2. Titration of D-antigen amount

D-antigen units in the sIPVs were measured by a sandwich ELISA method [12] in which D-antigen-specific mouse monoclonal antibodies were used as capture antibodies, and poliovirus (types 1–3)-specific rabbit antibodies were used as detector antibodies. A D-antigen reference for each type of sIPV was calibrated using the WHO S-IPV as reference (91/672). The amount of D-antigen units in each vaccine was measured against a D-antigen reference in a parallel line assay.

2.3. Animal study

Male *M. fascicularis* aged eight months were used for this study. The monkeys were divided into three test groups of three monkeys per group such that the total body weight in each group was almost the same.

The primary immunization consisted of three administrations of 0.5 mL of vaccine A, B, or C inoculated subcutaneously at 3-week intervals in the backs of monkeys, and was followed by one booster at 2.5 months after the third injection. Blood was collected from each monkey before inoculation and 3 weeks after each inoculation (Fig. 1).

2.4. Preliminary clinical study

The immunogenicity and safety of Vaccine A was evaluated in 85 healthy children (age range: 3–6 months) using the licensed DTaP vaccine, "KAKETSUKEN" Syringe as the control (Clinical trials registration: JapicCTI-121942). This was a multicenter, randomized, double-blind, parallel-group study during the primary immunization phase. After informed consent was obtained, eligible subjects were randomly assigned to two groups that received Vaccine A ($n = 42$) or DTaP vaccine as the control ($n = 43$). For the primary immunization, vaccine A or DTaP vaccine was administered subcutaneously at a dose of 0.5 mL once every 3–8 weeks, a total of three times. After finishing the primary immunization, the code was broken. And then, OPV was administered in the control group at a dose of 0.05 mL a total of two times (each at an interval of more than 6 weeks), followed by one booster (Vaccine A or DTaP vaccine) at 6–18 months after the primary immunization. Blood was collected at 4–8 weeks after the immunization.

This clinical study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, GCP, and relevant regulatory laws.

2.5. Titration of antibody

The sera were titrated for neutralizing antibody against attenuated poliovirus strains (Sabin-1, Sabin-2, and Sabin-3) and virulent poliovirus strains (Mahoney, MEF-1, and Saukett), and diphtheria toxin, and for anti-tetanus antibody, using a passive agglutination immunoassay. The titer of neutralizing antibody against each poliovirus was measured by a neutralization test method using HEp2 cells [13]. The titer of neutralizing antibody against the diphtheria toxin was measured by a neutralization test method using Vero cells [14]. The anti-tetanus antibody was measured using the KPA kit (including artificial particles sensitized with highly purified tetanus toxoid) manufactured by Kaketsuken. This test was carried out in accordance with the manufacturer's instructions.

3. Results

3.1. Titers of neutralizing antibody against polioviruses

M. fascicularis aged eight months were inoculated subcutaneously three times at 3-week intervals in the back with vaccines A, B, or C in doses of 0.5 mL, followed by one booster at 2.5 months after the third inoculation (Fig. 1).

Figs. 2 and 3 show plots of the titers of neutralizing antibody against the attenuated and virulent poliovirus strains from pre-inoculation to the fourth inoculation of Vaccines A, B, and C, respectively. The titers of neutralizing antibody against the attenuated and virulent poliovirus strains increased depending on the number of inoculations from pre-inoculation to the third

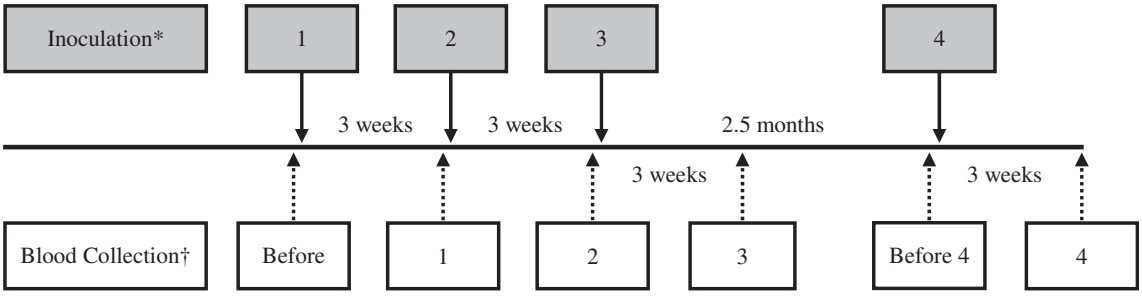

Fig. 1. Schedule of inoculation and blood collection in the animal study. * Numbers in gray boxes represent the number of inoculations. The primary immunization consisted of three administrations of 0.5 mL of vaccine A, B, or C inoculated at 3-week intervals, and was followed by one booster at 2.5 months after the third injection. † Numbers in white boxes represent the timing of blood collection. "Before" indicates before the first inoculation. Number "1" indicates collection at three weeks after the first inoculation; "2" indicates collection at three weeks after the second inoculation; "3" indicates collection at three weeks after the third inoculation; "before 4" indicates a time period before the fourth inoculation; and "4" indicates collection at three weeks after the fourth inoculation.