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A national reference for inactivated polio vaccine derived from Sabin strains in Japan

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ABSTRACT

As one aspect of its campaign to eradicate poliomyelitis, the World Health Organization (WHO) has encouraged development of the inactivated polio vaccine (IPV) derived from the Sabin strains (sIPV) as an option for an affordable polio vaccine, especially in low-income countries. The Japan Poliomyelitis Research Institute (JPRI) inactivated three serotypes of the Sabin strains and made sIPV preparations, including serotypes 1, 2 and 3 D-antigens in the ratio of 3:100:100. The National Institute of Infectious Diseases, Japan, assessed the immunogenic stability of these sIPV preparations in a rat potency test, according to an evaluation method recommended by the WHO. The immunogenicity of the three serotypes was maintained for at least 4 years when properly stored under −70 °C. Based on these data, the sIPV preparations made by JPRI have been approved as national reference vaccines by the Japanese national control authority and used for the quality control of the tetracomponent sIPV-containing diphtheria–tetanus–acellular pertussis combination vaccines that were licensed for a routine polio immunization in Japan.

1. Introduction

Poliomyelitis is caused by polioviruses of the family *Picornaviridae*. This neurological disorder manifests clinically as acute flaccid paralysis [1,2]. No antiviral drugs against polioviruses are commercially available, but vaccines have been very effective in preventing and controlling epidemics. Since the World Health Organization (WHO) launched the global polio eradication program in 1988, the number of patients with poliomyelitis caused by wild polioviruses have steadily decreased worldwide from approximately 350,000 cases in 1988 to 406 cases in 2013 [3].

Oral polio vaccines (OPVs) have been particularly important for curtailing the epidemic of poliomyelitis. These effective and safe tools have several key advantages. They are inexpensive to produce, easy to administer, and induce much better mucosal immunity than inactivated polio vaccines (IPVs). In polio-free areas, however, concerns have been raised about the use of OPVs as a live vaccine. For example, risk of vaccine-associated paralytic poliomyelitis (VAPP) is small but not insignificant [4], and polio outbreaks caused by vaccine-derived polioviruses (VDPVs), highly neurovirulent OPV-derived variants, can occur [5]. Thus, many countries that have eradicated wild polioviruses have adopted IPVs to minimize the risks of VAPP and polio outbreaks caused by VDPVs.

Abbreviations: $CCID_{50}$, 50% cell culture infective dose; cIPV, conventional inactivated polio vaccine; DTaP, diphtheria–tetanus–acellular pertussis combination vaccine; DU, D-antigen unit; IPV, inactivated polio vaccine; JPRI, Japan Poliomyelitis Research Institute; NIID, National Institute of Infectious Diseases, Japan; OPV, oral polio vaccine; sDU, D-antigen unit for Sabin vaccine; sIPV, Sabin-derived inactivated polio vaccine; SO, Sabin Original; VAPP, vaccine-associated paralytic poliomyelitis; VDPV, vaccine-derived polioviruses; WHO, World Health Organization.

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Until quite recently, only conventional IPVs (cIPVs) were used to prevent poliomyelitis. However, cIPVs are produced from virulent wild polioviruses, representing that much attention should be paid so as not to be a potential source of new infections. Thus, WHO has encouraged the development of IPVs derived from the Sabin strains (sIPVs) for as an alternative to cIPVs, especially in low-income countries during the final stage of global polio eradication [6]. After polio eradication, wild polioviruses should be quarantined [6–9].

The attenuated mutant Sabin strains are much less neurovirulent than wild strains and have been used as the source of OPVs. It is therefore possible that they are safer than the wild strains and more suitable for production of IPVs. In the light of the VDPV outbreaks and VAPP cases, it is not yet clear how much safer they will be in practice. In addition, careful risk assessment will be needed for an appropriate containment of Sabin strains during the process for manufacturing sIPVs in the post-eradication era. It is assured that sIPVs induce neutralizing antibodies against the wild virulent poliovirus strains [10,11], indicating that sIPVs have adequate potency to be substituted for cIPVs.

Worldwide three institutes have developed their own sIPVs: the Japan Poliomyelitis Research Institute (JPRI) in Japan, Intravacc (formerly part of National Institute for Public Health and the Environment and Netherlands Vaccine Institute), and the Chinese Academy of Medical Sciences in Kunming, China. Responding to a call from WHO for new polio vaccines, Intravacc developed a robust and transferable production process for sIPV. They have been promoting standardization of the quality control for sIPVs and attempting to offer technologies for sIPV production to low- or middle-income countries [12–14].

JPRI, a manufacturer of a Sabin OPV, made sIPV preparations, including all three serotypes of polioviruses in the D-antigen unit (DU) ratio of 3:100:100 (serotypes 1, 2 and 3, respectively) [15]. The first-generation preparations included 3, 100 and 100 sDU/0.5 mL for serotypes 1, 2 and 3, respectively, so that the immunogenicity of the respective serotypes in rats is nearly equal to that of cIPVs (containing 40, 8 and 32 DU/0.5 mL for serotypes 1, 2 and 3, respectively). However, it should be noted that the DU for sIPVs is not equivalent to that for cIPVs, which has been clearly shown by Simizu et al. [15]. That is, the DUs of the WHO cIPV reference (91/574) whose indicated titers were 430, 95 and 285 DU/mL for serotypes 1, 2 and 3, respectively [16], were measured as 96.2, 150 and 549 sDU/mL by in-house ELISA using anti-Sabin antibodies prepared at JPRI with each serotype of Sabin strains used as a standard substance (see Section 2 for the method of in-house ELISA). Note that "sDU" represents the D-antigen unit for sIPVs and will be used in this manuscript in order to distinguish between cIPV and sIPV and avoid misleading. This discrepancy is possibly because different antibodies and standards are used for D-antigen ELISA of sIPV and cIPV. This is also possibly because of different antigenic structure between sIPV and cIPV. In this context, to measure the D-antigen content of sIPV products from various sources, the standardization of assaying the D-antigen content and the establishment of International Reference Reagents for sIPVs are currently under investigation by the WHO Technical Working Group [17].

Here we report our evaluation of the stability of sIPV preparations made by JPRI. We monitored the D-antigen content and immunogenicity of each serotype in rats over a long period. Based on the results, the sIPV preparations were approved as national reference vaccines by the Ministry of Health, Labour and Welfare of the Japanese government, and used for quality control of the tetracomponent vaccines consisting of sIPV and a tricomponent diphtheria–tetanus–acellular pertussis combination antigen (DTaP-sIPV). Currently, two DTaP-sIPV products from the Chemo-Sero-Therapeutic Research Institute (Kaketsuken) and the Research Foundation for Microbial Diseases of Osaka University (Biken) were licensed for the production in 2012 and used for a routine polio immunization for infants and young children in Japan.

Table 1
The D-antigen contents in the sIPV preparations.

sIPV Lot	Date of preparation	D-antigen contents (sDU/0.5 mL)		
		Type 1	Type 2	Type 3
04C	Aug. 11, 2004	3.9	113	112
05J	Oct. 20, 2005	2.7	105	108
09A	Jul. 15, 2009	5.5	176	201
12A	Jul. 4, 2012	5.7	199	192

The D-antigen contents were determined by a sandwich ELISA method against the in-house reference standard of the Sabin polioviruses as described in Section 2 and were expressed as the Sabin D-antigen unit (sDU).

2. Materials and methods

2.1. Preparation of sIPV

sIPV bulk preparations were prepared as described [15]. The following Sabin strains were used: the LSc 2ab strain for serotype 1, the P712,Ch,2ab strain for serotype 2, and the Leon $12a_1b$ strain for serotype 3. The respective virus master seeds were established from the WHO/Behringwerke 1976 (SO+1 = Sabin Original + 1 passage) for serotype 1, the WHO/Behringwerke 1976 (SO+1) for serotype 2, and the Leon $12a_1b$/KP3 (SO) for serotype 3 [17]. Each strain was independently propagated from its working seed in Vero cells that were purchased from the American Type Culture Collection (CCL-81). After purification, the virus-containing fluid was inactivated by treating with 0.025% formalin for 12 days at 37 °C. Each serotype of monovalent sIPV was mixed with the others to obtain the trivalent sIPV preparations. The D-antigen contents of Lots 04C, 05J, 09A, and 12A of sIPV preparations manufactured by JPRI are shown in Table 1. sIPV bulk preparations were produced under conditions of good manufacturing practice in essentially the same way as a routine production process for cIPV [18].

2.2. D-antigen ELISA

A D-antigen ELISA was performed as described [15,19]. The wells of 96-well microtiter plates (Nunc Immunoplate Maxisorp, Thermo Fisher Scientific, Waltham, MA) were coated with D-antigen-specific mouse monoclonal antibodies against each serotype of Sabin strains (MA107-8W, MA201-159 and MA303-182L for serotypes 1, 2 and 3, respectively), which were isolated by JPRI [15,19]. Antibodies were diluted by a factor of 20,000 in 0.05 M sodium carbonate buffer. The plates were incubated for 3 h at 36 °C and washed three times with a wash buffer (0.01 M PBS containing 0.05% Tween 20). Samples of sIPV preparations in a dilution buffer (0.01 M PBS containing 0.05% Tween 20 and 1% BSA) were placed on wells in line B on the plate, followed by serial twofold dilutions to line G. The plates were incubated overnight at 4 °C and washed three times. Fifty microliter of serotype-specific neutralizing rabbit antibodies (dilution factor for serotypes 1 and 3: 1000; and serotype 2: 2000) were added to each well on the respective plates, and the plates were incubated for 1 h at 36 °C. After washing the plate, 50 μL of anti-rabbit IgG conjugated with horseradish peroxidase (0.21 μg of protein/mL, ICN/Cappel, Aurora, OH) were added to each well, and the plates were incubated for 1 h at 36 °C. The *o*-phenylenediamine chromogen and hydrogen peroxide substrate were added, and the plates were incubated for 20 min at 36 °C. The reaction was stopped by adding 50 μL of 2 M H_2SO_4, and the absorbance at 492 nm was measured. sDUs in samples were calculated by the parallel line method [20] against an in-house reference standard virus whose sDUs were adjusted