Intestinal antibody responses to two novel live attenuated type 2 oral poliovirus vaccines in healthy adults in Belgium

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40 Word Summary: In a phase 1 trial, two novel type 2 oral polio vaccine candidates induced detectable poliovirus type 2-specific intestinal neutralizing responses in 40.0% and 46.7% of participants respectively, suggesting potential utility for reducing circulating vaccine-derived poliovirus transmission under outbreak scenarios.

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Abstract
In a blinded phase 1 trial (EudraCT 2017-0000908-21; NCT03430349) in Belgium, healthy adults (18 to 50 years) previously immunized exclusively with inactivated polio vaccine were administered a single dose of one of two novel type 2 oral polio vaccines (nOPV2-c1: S2/cre5/S15domV/rec1/hifi3 (N=15); nOPV2-c2: S2/S15domV/CpG40 (N=15)) and isolated for 28 days in a purpose-built containment facility. Using stool samples collected near days 0, 7, 14, and 28, we evaluated intestinal neutralization and IgA responses to the novel OPV2s and found that nOPV2-c1 and nOPV2-c2 induced detectable poliovirus type 2-specific intestinal neutralizing responses in 40.0% and 46.7% of participants respectively.

Keywords: poliovirus, live attenuated vaccine, mucosal immunity, intestinal antibodies, eradication
Background

The world is approaching the eradication of polio. The certification on 25 August 2020 that the WHO African Region has achieved wild-type poliovirus-free status marks an important milestone in the endgame. However, Afghanistan and Pakistan continue to experience local transmission with newly detected cases of wild-type poliovirus type 1. Of equal concern, more than 20 countries have reported cases with circulating vaccine-derived polioviruses (cVDPVs) in 2020.

Type 2 cVDPVs present a notable challenge to eradication efforts. In April-May 2016, the routine use of the type 2 component of the Sabin oral polio vaccine (OPV2) was stopped globally, and the inactivated Salk polio vaccine (IPV) became the primary public health tool for inducing immunity to poliovirus type 2. The aims of removing OPV2 from routine vaccination were to limit new emergences of type 2 cVDPVs, minimize risks of vaccine-associated paralytic poliomyelitis (VAPP), and enhance immunogenicity of the bivalent OPV (bOPV) against poliovirus types 1 and 3 with which OPV2 interfered. Both Sabin and Salk vaccines are capable of inducing robust serum immune responses that can limit viremia, inhibit entry into the central nervous system, and protect vaccinated individuals from paralytic polio. However, primary vaccination schedules based on inactivated vaccine, in contrast to live attenuated vaccine, fail to induce intestinal mucosal immune responses that are critical for inhibiting enteric poliovirus replication and preventing fecal-oral transmission [1-6]. The cessation of the routine use of OPV2 has therefore led to a population level decrease in intestinal mucosal immunity to type 2 polioviruses, which may facilitate the continued circulation of type 2 VDPVs, especially in regions with sub-optimal IPV coverage. In outbreak scenarios where communities are experiencing rapid spread of type 2 cVDPVs and risks of paralysis, reintroduction of Sabin monovalent OPV2 has thus been the only recourse for halting transmission. However, even in highly regulated short-term campaigns,
monovalent OPV2 has the potential to genetically mutate and seed further cVDPV outbreaks, perpetuating a vicious cycle that threatens eradication.

To counter the limitations of the Sabin OPV2, recent efforts have focused on developing novel OPV2 (nOPV2) candidates that are engineered to be more genetically stable and less likely to revert to a neurovirulent form. A 2017 blinded phase 1 trial in Belgium of healthy adults provided encouraging evidence of the safety and systemic immunogenicity of two nOPV2 candidates (nOPV2-c1: S2/cre5/S15domV/rec1/hif13; nOPV2-c2: S2/S15domV/CpG40) [7]. Although both candidates induced serum neutralizing antibodies, the median duration and magnitude of nOPV2 viral shedding was approximately 2-times higher for nOPV2-c1 than nOPV2-c2. Using stool samples collected serially from the 15 participants per candidate (30 in total) in this trial, this study aims to investigate the intestinal mucosal immune responses observed after oral administration of these nOPV2 candidates.

Methods

Study design and participants

The study design, candidate vaccines, and outcomes of the parent clinical trial have been reported in detail [7]. Briefly, healthy volunteers, aged 18 to 50 years who were previously immunized exclusively with IPV (up to 6 doses), were recruited between 22 May and 22 August 2017 to participate in a blinded phase 1 clinical trial (EudraCT 2017-0000908-21; NCT03430349) of two novel live attenuated OPV2s with a 28-day isolated follow-up in a purpose-built containment facility, referred to as Poliopolis [8], at the University of Antwerp (Antwerp, Belgium). The vaccine candidates are monovalent live-attenuated type 2 polioviruses derived from a modified Sabin-strain type 2 infectious cDNA clone and propagated in Vero cells; both candidates were engineered to improve the genetic stability of their attenuation (for further details, see [7]).
Following a medical and psychological screening, eligible participants were sequentially allocated into two groups based on the sealed envelope selection of the first participant, with the first 15 participants receiving nOPV2-c2 and the second 15 participants receiving nOPV2-c1. Participants were closely monitored for solicited and unsolicited adverse events. Blood samples were collected on days 0 and 28. Nasopharyngeal swab samples were collected on days 0, 3, 7, and the final day of containment. Stool samples were collected daily as possible.

**Laboratory procedures**

As part of the parent clinical trial, investigators at the Centers for Disease Control and Prevention (Atlanta, GA, USA) evaluated poliovirus type 2-specific neutralizing antibody responses in serum and nOPV2 virus shedding in nasopharyngeal swab and stool samples [7]. Titers of neutralizing antibodies were measured in sera using standardized procedures [9]. Poliovirus type 2 RNA was detected in nasopharyngeal swab and stool suspensions using a Sabin multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) assay [10]. The magnitude of infectious virus in RT-PCR-positive samples was measured as the CCID\textsubscript{50} per gram of stool using a modified WHO cell sensitivity assay as previously described [9].

For the current study, investigators at Dartmouth-Hitchcock Medical Center (Lebanon, NH, USA) measured poliovirus serotype-specific intestinal neutralizing activity and immunoglobulin A (IgA) binding in the same stool samples using methods as previously described [1]. Briefly, stool neutralizing activity was measured by limiting dilution inhibition of luciferase-expressing wild-type-derived polio pseudoviruses [11] and expressed as the titer needed to achieve 60% neutralization (titers <4 were considered undetectable and recorded as 2). Total and poliovirus type-specific IgA in stool were quantified using a multiplex
microsphere assay developed by coupling monovalent IPVs to fluorescently coded magnetic microspheres.

**Ethics**

At enrollment, all participants provided written informed consent, which included provisions for the use of samples in future polio-related studies. Ethical approval was provided by the hospital and university institutional review boards of Dartmouth-Hitchcock Medical Center, the University of Antwerp, and the Antwerp University Hospital.

**Results**

Poliovirus type-specific intestinal antibody responses were evaluated in 30 adult participants (83% male; mean age (SD): 32.3 (9.4) years) using 94 stool samples from the 15 nOPV2-c1 recipients and 95 stool samples from the 15 nOPV2-c2 recipients. IgA was detected in stool from all participants, and the median concentration of total IgA in the first pre-vaccination stool was 9440 ng/mL (IQR: 1620 to 22,500). Whereas all participants had documented poliovirus type 2-specific serum neutralization on day 0 (median titer (IQR): nOPV-c1=56.9 (36.0, 181); nOPV2-c2=36.0 (22.6, 90.5)), only one participant had detectable poliovirus type 2-specific stool neutralization (i.e., with a titer of 4) at baseline (Table).

Following vaccination, nOPV2 RNA was detected in stool from 100% (15/15) of participants who received nOPV2-c1 and from 87% (13/15) of participants who received nOPV2-c2, as previously reported [7]. In contrast, poliovirus type 2-specific intestinal neutralizing responses in stool were detected (i.e., with a titer >2 at ≥1 timepoint) after vaccination in only 40% (6/15) of nOPV2-c1 recipients and 47% (7/15) of nOPV2-c2 recipients (Table, Supplementary Figure 1). No type 2-specific intestinal neutralizing responses were detected in the two participants with no detectable nOPV2 shedding.
Baseline serum neutralizing titers did not appear to correlate with subsequent stool neutralizing responses to the vaccines (Supplementary Figure 2).

Among participants with detectable poliovirus type 2-specific intestinal neutralization, the observed neutralizing antibody titers in stool were modest (Figure). Specifically, the geometric means of the peak poliovirus type 2-specific stool titers (i.e., the highest titer observed per participant over the duration of follow-up) were 16.8 (range: 4 to 166) for the 6 participants with detectable intestinal neutralizing responses to nOPV2-c1 and 27.2 (range: 7 to 84) for the 7 participants with detectable intestinal neutralizing responses to nOPV2-c2. Whereas the peak titers of poliovirus type 2-specific stool neutralization and IgA were closely correlated with each other (Spearman’s rho: nOPV2-c1=0.69, p=0.005; nOPV2-c2=0.62, p=0.013), neither indicator correlated significantly with the peak magnitudes of vaccine virus shedding (p>0.05 for both) (Supplementary Figure 1). Although not consistently statistically significant based on Spearman’s correlations, scatterplots suggest a potential positive trend between day 28 poliovirus type 2-specific serum neutralization and intestinal immune responses (Supplementary Figure 2). There was also evidence of a correlation of peak poliovirus type 2 stool IgA with peak IgA levels for poliovirus types 1 (Spearman’s rho: nOPV2-c1=0.65, p=0.009; nOPV2-c2=0.77, p=0.0007) and 3 (Spearman’s rho: nOPV2-c1=0.38, p=0.16; nOPV2-c2=0.76, p=0.001).

Discussion

In this Phase 1 clinical trial in a population of healthy IPV-immunized adults, we observed a modest but detectable rise in poliovirus type 2-specific intestinal mucosal neutralizing activity and IgA antibodies after administration of the two nOPV2 candidates. Despite a longer duration and higher magnitude of shedding in nOPV2-c1 versus nOPV2-c2 [7], the mucosal type 2-specific immune responses, like the serum neutralizing antibody responses [7], did not clearly differentiate the performance of the two vaccine candidates.
among the small number of participants evaluated in this study. nOPV2-c1 and nOPV2-c2 similarly induced detectable poliovirus type 2-specific intestinal neutralizing responses in 40.0% and 46.7% of participants respectively. Peak titers of poliovirus type 2-specific stool neutralization and IgA correlated with each other but were not associated with the peak magnitudes of vaccine virus shedding.

These observations contribute to our growing understanding of vaccine-induced mucosal immunity to poliovirus across the life course. In young infants, primary vaccine series using only IPV induce negligible intestinal mucosal immunity to poliovirus [2, 3], and replication of Sabin OPV virus is not limited upon subsequent challenge [12]. In contrast, vaccine schedules using OPV in infancy induce strong intestinal mucosal antibody responses and largely sterilizing immunity on OPV challenge [1]. Although there is evidence that OPV-induced intestinal immunity may wane significantly within a year of vaccination [13], a study of adults, aged 20 to 44 years, reported detecting mucosal IgA antibodies against all three serotypes of poliovirus in stool from 3 of the 11 participants who received OPV in childhood [14]. Further, an investigation in adolescents, aged 16 to 18 years, found evidence of markedly lower frequencies of vaccine viral excretion in stool after OPV challenge in individuals who received OPV instead of IPV in childhood (percentage of recipients excreting OPV types 1, 2, and 3 after challenge: OPV (with or without IPV) history = 20%, 24%, and 30% versus IPV-only history = 50%, 69%, and 50% [15].

Building on this work, two recent investigations suggest the induction of mucosal immunity may be diminished if OPV is delivered beyond infancy. In a study of exclusively IPV-vaccinated children, aged 1 to 5 years, in Lithuania who were challenged with one or two doses of mOPV2, only 32% of evaluable participants achieved a type 2-specific stool neutralizing titer of ≥32 after the first dose of mOPV2 [6]. Despite having been previously vaccinated with one dose of mOPV2 and at least three doses of IPV, 32 of the 47 (68%)
children receiving two doses of mOPV2 were reported to excrete virus following receipt of the second mOPV2 dose. Further evidence comes from an mOPV1 challenge study in adults, aged 18 to 50 years, in Sweden who were vaccinated with four doses of IPV in childhood. Although each of the 12 evaluated participants had documented mOPV1 shedding following challenge, poliovirus type 1-specific neutralizing activity in stool samples remained low, never exceeding a titer of 18.4 for any of the participants over follow-up [4].

Although no direct comparisons of the responses can be made and it remains possible that the nOPV2 candidates may induce lower levels of mucosal antibodies than the original Sabin OPV strains, the modest intestinal antibody responses observed in the current Phase 1 trial in healthy adults are also consistent with our hypothesis of an age-related diminution in the mucosal immunogenicity of live attenuated polio vaccines. Further research is needed to define the differences in mucosal immune responses to vaccination in children and adults. Potential mechanisms that warrant further investigation include oral tolerance, age-related immunosenescence, and an influence of IPV as initial immunogen. Further, assay sensitivity in relation to age at stool collection remains an area of active investigation. The results from a subsequent Phase II trial (NCT03554798), which administered the nOPV2 candidates to healthy children, aged 1 to 5 years, previously vaccinated with bOPV-bOPV-bOPV+IPV in Panama will be critical for understanding the role of age in the mucosal immunogenicity of the nOPV2 candidates.

In conclusion, these results contribute to our broader understanding of the induction of mucosal immunity. The modest but clear induction of mucosal immune responses to nOPV2s suggest the vaccines are likely to reduce cVDPV transmission under outbreak scenarios and strengthen the case for further clinical trials.
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Conflicts of Interest: The authors declare no conflicts of interest.

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Table. Poliovirus type-specific serum and intestinal antibody responses to the nOPV2 candidates.

| Sample  | Immune Marker                              | Day | nOPV2-c1 (N=15) | nOPV2-c2 (N=15) | \(P\) value, Chi-squared or Mann-Whitney U |
|---------|--------------------------------------------|-----|-----------------|-----------------|-------------------------------------------|
| Serum   | PV type 2 serum neutralization titer       | 0   | 56.9 (36.0, 181) | 36.0 (22.6, 90.5) | 0.10                                      |
|         |                                            | 28  | 1150 (576, 1450) | 724 (362, 1150)  | 0.27                                      |
| Stool\(^a\) | Detectable PV type 2 stool neutralization\(^b\) | 0   | 1 (6.7%)        | 0 (0%)          | 0.31                                      |
|         |                                            | 14  | 4 (26.7%)       | 4 (26.7%)       | 1.00                                      |
|         |                                            | 21  | 3 (20.0%)       | 2 (13.3%)       | 0.62                                      |
|         |                                            | 28  | 5 (33.3%)       | 5 (33.3%)       | 1.00                                      |
|         |                                            | All | 6 (40.0%)       | 7 (46.7%)       | 0.71                                      |
| PV type 2 stool neutralization titer | 0   | 2 (2, 2)        | 2 (2, 2)        | 0.32                                      |
|         |                                            | 14  | 2 (2, 2.7)      | 2 (2, 2.5)      | 0.96                                      |
|         |                                            | 21  | 2 (2, 2.2)      | 2 (2, 2)        | 0.59                                      |
|         |                                            | 28  | 2 (2, 4)        | 2 (2, 7)        | 0.79                                      |
| PV type 2 stool IgA MFI      | 0   | 11.3 (2.0, 14.1) | 12.0 (3.5, 17.0) | 0.26                                      |
|         |                                            | 14  | 6.8 (3.2, 22.3) | 7.4 (5.6, 38.1) | 0.42                                      |
|         |                                            | 21  | 10.8 (5.0, 19.5) | 10.0 (2.5, 46.5) | 0.72                                      |
|         |                                            | 28  | 14.5 (2.0, 29.5) | 12.3 (1.5, 112) | 0.76                                      |
| PV type 1 stool IgA MFI      | 0   | 6.3 (2.0, 21.5) | 13.0 (5.9, 22.5) | 0.25                                      |
|         |                                            | 14  | 8.0 (1.5, 11.3) | 9.0 (4.4, 24.0) | 0.42                                      |
|         |                                            | 21  | 4.3 (0, 8.0)    | 3.5 (0, 16.0)   | 0.72                                      |
|         |                                            | 28  | 6.3 (0, 12.0)   | 4.5 (0, 28.0)   | 0.68                                      |
| PV type 3 stool IgA MFI      | 0   | 5.9 (3.5, 31.0) | 13.8 (2.0, 40.5) | 0.56                                      |
|         |                                            | 14  | 3.5 (0, 15.0)   | 8.3 (3.4, 20.0) | 0.16                                      |
|         |                                            | 21  | 0 (0, 4.8)      | 6.0 (0, 18.5)   | 0.15                                      |
|         |                                            | 28  | 2.0 (0, 14.5)   | 1.0 (0, 18.8)   | 0.73                                      |

Abbreviations: PV, poliovirus; IgA, immunoglobulin A; MFI, mean fluorescence intensity.

\(^a\) As stool samples were not available for all participants on all days, we described the aggregate of the measurements obtained from samples collected days -2 to 0 (pre-vaccination) for day 0, days 12 to 16 for day 14, days 19 to 23 for day 21, and days 26 to 30 for day 28. If two or more stool samples were collected within each window for a given participant, we calculated the geometric mean of the stool neutralization titer or IgA mean fluorescence intensity.

\(^b\) We considered any poliovirus type 2-specific stool neutralization titer >2 to be detectable.
Figure. Reverse cumulative distribution functions of poliovirus type 2-specific stool neutralizing activity and IgA levels before and after receiving (A, B) nOPV2-c1 (N=15) and (C, D) nOPV2-c2 (N=15). Abbreviations: IgA, immunoglobulin A; MFI, mean fluorescence intensity.

As stool samples were not available for all participants on all days, we described the aggregate of the measurements obtained from samples collected days -2 to 0 (pre-vaccination) for day 0, days 12 to 16 for day 14, days 19 to 23 for day 21, and days 26 to 30 for day 28. If two or more stool samples were collected within each window for a given participant, we calculated the geometric mean of the stool neutralization titer or IgA mean fluorescence intensity.
