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A highly immunogenic UVC inactivated Sabin based polio vaccine

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Despite their efficacy, the currently available polio vaccines, oral polio vaccine (OPV) and inactivated polio vaccine (IPV), possess inherent flaws posing significant challenges in the global eradication of polio. OPV, which uses live Sabin attenuated strains, carries the risk of reversion to pathogenic forms and causing vaccine-associated paralytic poliomyelitis (VAPP) and vaccine-derived polio disease (VDPD) in incompletely vaccinated or immune-compromised individuals. Conventional IPVs, which are non-replicative, are more expensive to manufacture and introduce biohazard and biosecurity risks due to the use of neuropathogenic strains in production. These types of limitations have led to a call by the Global Polio Eradication Initiative and others for the development of updated polio vaccines. We are developing a novel Ultraviolet-C radiation (UVC) inactivation method that preserves immunogenicity and is compatible with attenuated strains of polio. The method incorporates an antioxidant complex, manganese-decapeptide-phosphate (MDP), derived from the radioresistant bacterium *Deinococcus radiodurans*. The inclusion of MDP protects the immunogenic neutralizing epitopes from damage during UVC inactivation. The novel vaccine candidate, *ultra*IPV™, produced using these methods demonstrates three crucial attributes: complete inactivation, which precludes the risk of vaccine-associated disease; use of non-pathogenic strains to reduce production risks; and significantly enhanced yield of doses per milligram of input virus, which could increase vaccine supply while reducing costs. Additionally, ultraIPV™ retains antigenicity post-freeze–thaw cycles, a testament to its robustness.

The certification of global eradication of wild-type poliovirus types 2 and 3 (WPV2 and WPV3) by the World Health Organization (WHO) in 2015 and 2019, respectively, marked a significant victory in our collective fight against polio[1–5]. WPV1 infections have been limited in both number and geographical region with just two remaining countries, Pakistan and Afghanistan, reporting infections since the start of 2017[6–9]. The workhorse of eradication efforts, the oral polio vaccine (OPV), is inexpensive and stimulates robust immunity in most vaccinated people. However, during replication in the gut, the disease attenuation phenotype can be reversed, and pathogenic virus can be shed. Infection from vaccine-derived poliovirus (VDPV) has become an increasingly serious and widespread problem[10–12]. In the years 2021, 2022, and 2023, the WHO reported 343, 1751, and 1 case of vaccine-derived paralytic polio from cVDP1, cVDP2, and cVDP3, respectively[13]. Because of the infections due to VDPV and eradication of wtPV2, trivalent OPV has been largely replaced by bivalent OPV containing PV1 and PV3 components or monovalent vaccines. A variety of replacement vaccines are in use or under consideration, including improved IPVs, novel oral polio vaccines (nOPVs), virus-like particles, and others[14–16].

Inactivated polio vaccine (IPV) is a formalin-inactivated injectable product that stimulates robust systemic and partial gut immunity in vaccinated children[17–20]. Unlike OPV, IPV cannot cause VDPV, and it is used exclusively in numerous countries[21]. The majority of conventional IPVs (cIPV) are produced by treating purified, wild-type, neuropathogenic virus for 2–4 weeks with formalin, and some countries have approved a Sabin-based IPV (sIPV). As global eradication progresses, the use of pathogenic strains in the manufacturing process presents increasingly serious biohazard and biosecurity risks. Escape of the virus from manufacturing plants into the environment has been documented[22–24]. Such a breach of biosecurity could undo decades of eradication efforts if it led to infection in under-vaccinated populations in the future. Because of the risks associated with the virus

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remaining in the environment and a small, but concerning number of chronic shedders, the WHO predicts that continued vaccine pressure will be required for at least 2 or 3 decades after eradication[25,26]. These pressing challenges have inspired our quest for a safer, more cost-effective IPV alternative.

Multiple strategies for producing safer polio vaccines have been underway for several years. Early efforts to substitute attenuated Sabin for neuropathogenic strains discovered that formalin inactivation damages the primary neutralization epitope located on VP1 of PV1 Sabin[27–29]. Although some countries, such as Japan and China, have approved formalin-inactivated Sabin vaccines, others, such as the US, have not yet done so. More recently, alternative vaccines composed of either attenuated strains that are engineered to be more resistant to becoming pathogenic by mutation or recombination events or recombinant virus-like particles are in development to provide additional options[15,21,30–42]. nOPV, an engineered PV2 strain, is particularly interesting as it has been used since March 2021 and is the current PV2 strain used in OPVs. It is possible that the long-term safety and immunogenicity profiles of Sabin-based IPVs or new vaccines that use engineered attenuated strains will take many years to fully characterize and assess. Furthermore, recent reports indicate that the novel attenuated strains can evolve to cause poliomyelitis which calls into question the use of any attenuated polio vaccine. Therefore, it is prudent to continue with additional efforts that could result in improved inactivated polio vaccines.

In light of these challenges, we report here an alternative Sabin-based vaccine that is inactivated using a rapid irradiation process. Our collaborative team has developed a manganese-decapeptide-phosphate (MDP) antioxidant that protects surface amino acids from damage during exposure to various types of radiation, including gamma, x-rays, and UV light[43–51]. MDP was derived through analysis of the extreme radio-resistance of *Deinococcus radiodurans*. The bacteria accumulate manganese-peptide-phosphate complexes that protect DNA repair enzymes from radiation damage. This innovative approach allows the separation of desired nucleic acid damage from undesired surface epitope damage. While the use of radiation and UV light has been applied to sterilization of both medical equipment and vaccine development, MDP is the key factor that dramatically increases the immunogenicity of radiation-inactivated viruses and bacteria, setting a new paradigm in vaccine development.

Previous efforts to develop an IPV using gamma irradiation faltered when conditions could not be established for the production of immunogenic PV3 Sabin component[50]. The UVC-inactivated novel vaccine candidate described here, *ultra*IPV™, sidesteps the use of neuropathogenic viruses in the manufacturing process, reduces the time required for inactivation, and produces more vaccine units per milligram of virus stock. Preclinical evaluations of our trivalent vaccine candidate in the Wistar rat, a recognized model for IPV immunogenicity studies, indicate that ultraIPV™ stimulates robust virus-neutralizing antibodies. In addition, the irradiation method is a platform technology that could facilitate a rapid response to emerging pathogens or improve the immunogenicity of other chemically-inactivated virus vaccines.

Results

Attenuated polio viruses are rapidly inactivated using UVC light

The formalin-inactivation process used for standard IPV products requires careful maintenance of temperature, pH, and other conditions over many days or weeks. The UVC-inactivation process is rapid, straightforward, and requires no elaborate equipment. Figure 1 shows typical UVC inactivation curves for the three Sabin viruses compounded with the MDP protectant using an inexpensive light wand emitting 5 mW/cm^2 for up to 21 s of exposure. Each of the three viruses demonstrated similar inactivation kinetics with inactivation rates of approximately 71% per second at 5 mW/cm^2. No infectivity could be detected after 15 or 18 s of exposure (75–90 mJ) when inactivated at concentrations of ~100 µg/mL. Following UVC-inactivation, the samples can be chromatographed in a size-exclusion column or washed in filtration cartridges (100,000 MWCO) to remove MDP components and increase the purity, if needed.

MDP protects the immunogenicity during UVC inactivation

Polio D antigen content provides a prediction of vaccine potency[52]. Standard IPV products are produced from wild-type strains and are formulated to contain 40 D antigen units of PV1, 8 of PV2, and 32 of PV3. Prior to the analysis of immunogenicity in an animal model, the D antigen content of the UVC-inactivated virus preparations was measured using a standard ELISA. Figure 2 shows D antigen ELISA titration curves of the three Sabin viruses after exposure to 120 mJoules UVC light (approximately 24 s at 5 mW/cm^2). As expected, irradiation of PV1 and PV2 Sabin viruses without the MDP protectant resulted in loss of D antigen concentration. Unexpectedly, PV3 Sabin appeared to retain D antigen concentrations whether or not MDP was used during UVC inactivation of the virus. The D antigen ELISA data were used to determine the antigen concentration of each inactivated virus for equilibration to conventional IPVs prior to immunization studies.

Extended irradiation

As reported by Plotkin and Orenstein, a small number of early batches of IPV were incompletely inactivated at Cutter Laboratories and tragically led to infection, paralysis, and death in a small number of vaccinees, now referred to as "The Cutter Incident"[53]. For this reason, the WHO has published guidelines for the analyses of inactivated polio vaccine to reduce the risk of releasing a vaccine lot that contains residual infectivity[54–56]. Briefly, test viruses are applied to susceptible cell monolayers, which are examined for CPE after 3–5 days. If no CPE is observed, the cells are freeze-thawed to release potential low-level viruses, and the lysed samples are used to inoculate a second round of indicator cells. A lack of CPE after five sequential passages indicates that no replication-competent virus was present in the initial inocula. Figure 3 shows such an analysis of UVC-

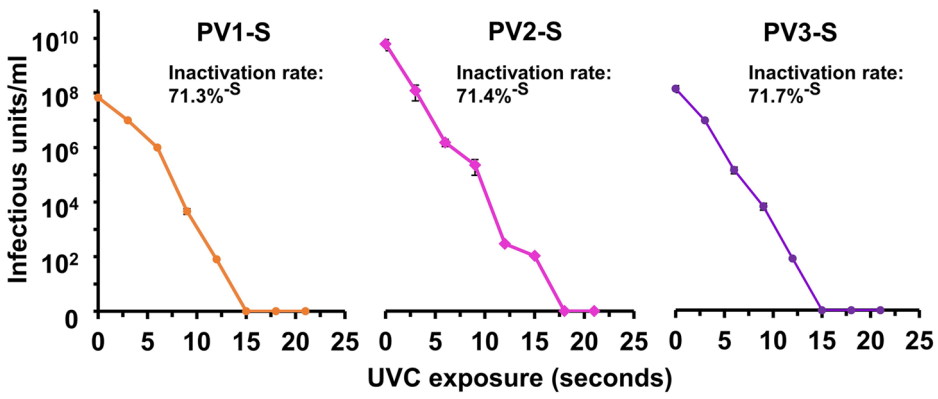

Fig. 1 | Decrease in infectivity of PV treated with UVC irradiation. $TCID_{50}$ virus titers are graphed vs. seconds of exposure to a UVC lamp outputting 4.8 mW/cm^2. Error bars are one standard deviation above and below the means. The *Y*-axis divisions are in Log-10 infectivity units. The zero point reflects no detectable infectivity and is not to be confused with $0log_{10}$.

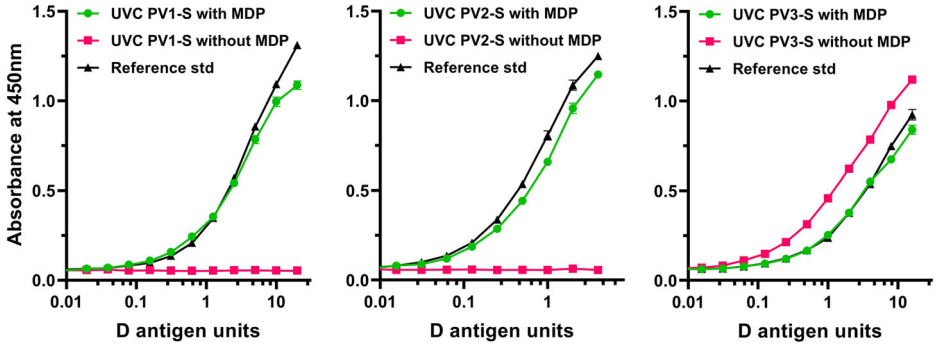

Fig. 2 | D antigen determination of PV1-S, PV2-S and PV3-S preparations UVC inactivated with and without the MPD complex present. In this experiment, MDP-containing samples were normalized to the D antigen content of a reference standard (IPOL) and samples irradiated without MDP are shown for comparison.

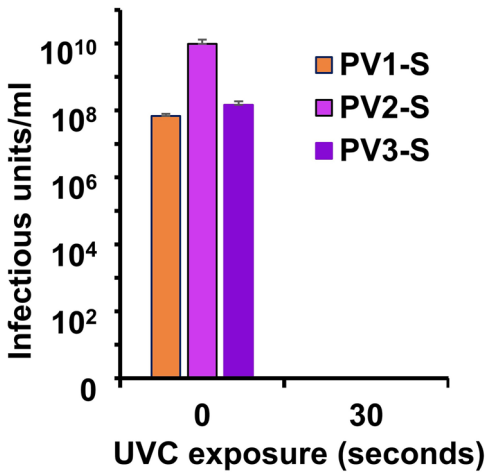

Fig. 3 | Analysis of potential low-level residual infectivity. PV1-, PV2-, and PV3-Sabin strains were complexed with the MDP protectant and exposed to 120 mJ UVC irradiation. The irradiated preparations were placed on MRC-5 indicator cells which were passaged five times to detect potential infectivity by CPE. No infectivity could be detected.

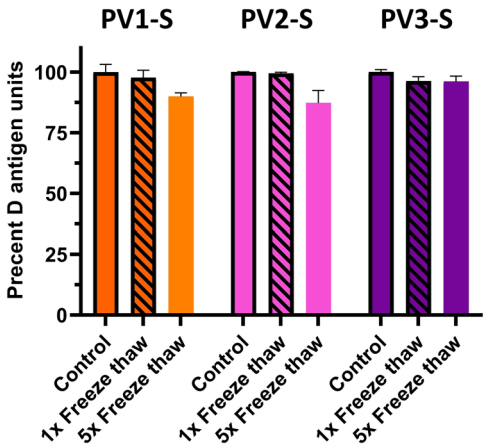

Fig. 4 | Stability of immunogenicity after freeze-thaw. D antigen concentrations were determined after 0, 1, and 5 rounds of freeze–thaw cycles.

inactivated PV1, PV2, and PV3 Sabin viruses that were exposed to 5 mW/cm^2 for 30 s (150 mJ).

Freeze-thaw stability

Both IPOL^R and VeroPOL^R require storage at 4 °C with shelf-lives of 3 and 2 years, respectively. The vaccine circulars that accompany the products warn against freezing the vials. Because it is possible that some agencies and organizations may want to store stockpiles of *ultra*IPV^{TM} for many years, we examined antigenic stability after freeze–thaw cycles. Using −80 °C as an overnight storage temperature, lots of *ultra*IPV^{TM} were subjected to either one or three freeze-thaw cycles and then tested for D antigen activity. Similar to those reported with formalin-inactivated sIPV[57], no appreciable loss of D antigen concentrations were observed after one freeze–thaw cycle and only minor losses were observed after three cycles (Fig. 4).

D antigen content per milligram of virus

The D-antigen assays used to formulate IPV vaccines utilize antibodies to neutralizing epitopes to measure antigenic content. The ratio of D-antigen units (DU) to virus mass (micrograms) may vary between products and vaccine lots. We determined the D antigen content of *ultra*IPV^{TM}, IPOL^R, and VeroPol^R using wild-type reference standards. One dose was defined as containing 40, 8, and 32 D antigen units of PV1, PV2, and PV3 components, respectively, for each of the three preparations. We then used mass spectroscopy (LC–MS/MS) to determine the viral protein content in micrograms per dose of PV1, PV2, and PV3 in *ultra*IPV^{TM} and the two conventional vaccines, IPOL^R and VeroPol^R. Total masses of VP1, VP2, VP3, and VP4 viral proteins were computed from the sum of the masses of the tryptic/ chymotryptic peptides. Table 1 shows the micrograms per dose based on the MS data. For example, each dose of *ultra*IPV^{TM} (40:8:32 DU formulation) contained 4, 0.04, and 2.2 µg of the Sabin strains of PV1, PV2, and PV3, respectively. These data were used to calculate the number of doses that can be produced per milligram.

Table 1 | MS-based analysis of doses per milligram of *ultra*IPV^{RTM}

Vaccine	Virus component	Micrograms per dose[a]	Doses per milligram
*Ultra*IPV^{TM} (Biological Mimetics, Inc.)			
	PV1	4	250
	PV2	0.04	25,000
	PV3	2.2	454
VeroPol^R (Statens Serum Institut)			
	PV1	67.5	14.8
	PV2	18	55.6
	PV3	54	18.5
IPOL^R (Sanofi Pasteur)			
	PV1	99.5	10.1
	PV2	55.2	18.1
	PV3	67.3	14.9

[a]Micrograms per dose determined by mass spectroscopy.

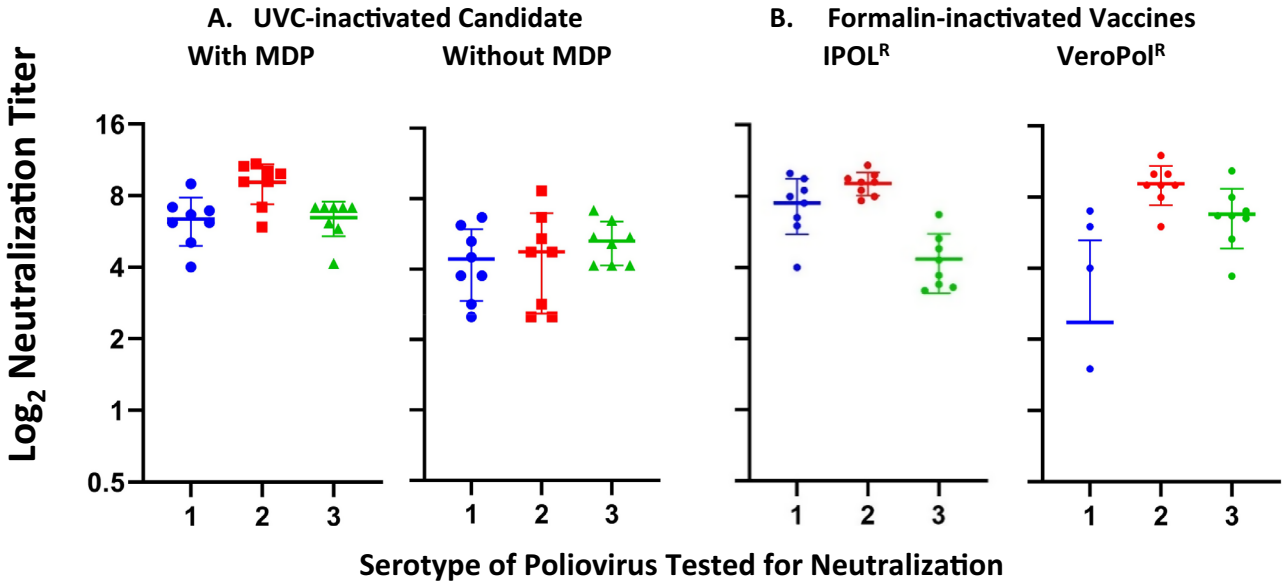

Fig. 5 | Log_2 virus neutralization titers of sera from rats immunized with one human dose of IPV defined as 40 D units PV1, 8 DU PV2, and 32 DU PV3. PV1, PV2, and PV3 viruses used in the neutralization assay are shown as 1, 2, and 3 on the *X*-axis. Panel **A** Neutralization titers from rats immunized with *ultra*IPV™. Panel **B** Titers from rats immunized with IPOL® (Left) and VeroPol® (Right).

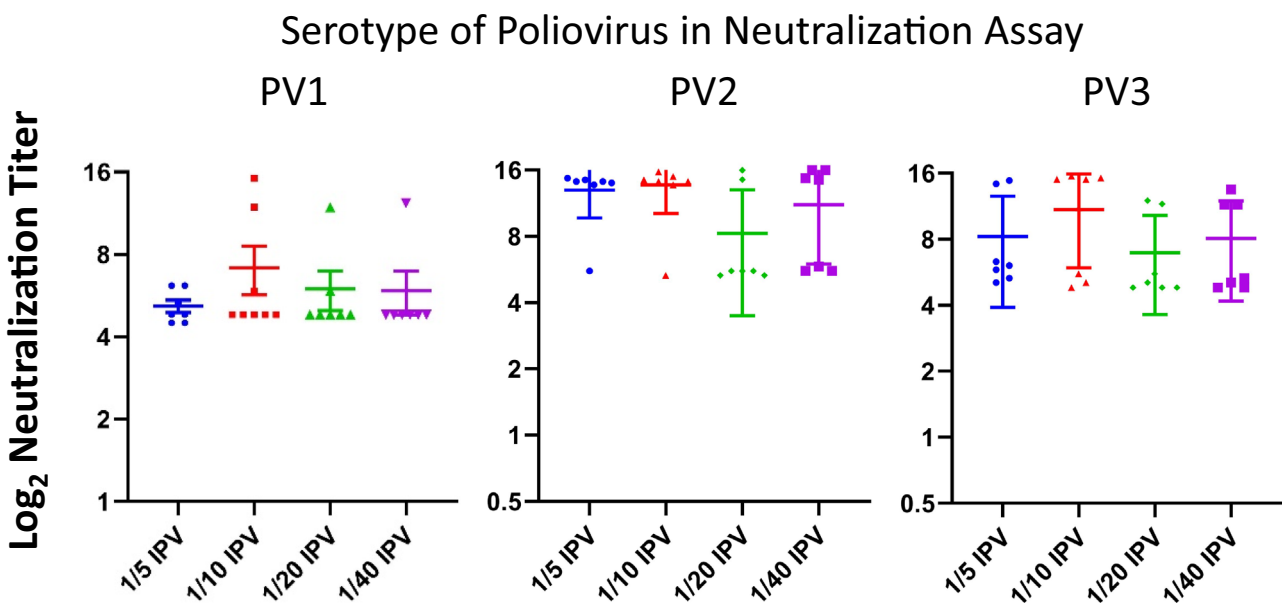

Fig. 6 | Neutralization titers of rat sera from fractional immunizations of *ultra*IPV™. Groups of 8 Wistar rats were immunized with fractional doses of *ultra*IPV™ in which one human dose is defined as 40:8:32 D antigen units of PV1, PV2, and PV3, respectively. Sera from individual rats were assayed for neutralization of the three serotypes using a standard $TCID_{50}$ assay. Mean neutralization titers are reported as horizontal lines with error bars showing one standard deviation.

The UVC-inactivated poliovirus vaccine candidate is highly immunogenic in Wistar rats

The accepted human poliovirus correlate of immune protection is the presence of neutralizing antibodies with titers of 1:8 (2^3) or higher[35,36]. For assessing the immunogenicity of IPV products, the Wistar rat has been an accepted model because its vaccine-elicited neutralizing titers closely predict human titers[34,35,37]. For the studies reported herein, the immunogen content of *ultra*IPV™ was adjusted to 40 D antigen units of PV1, 8 of PV2, and 32 of PV3 per dose[13,15,35,36]. Figure 5 presents the log-2 neutralization titers from Wistar rats immunized with two doses of either *ultra*IPV™, IPOL®, or VeroPol® delivered IM on Days 1 and 21, with the titers determined on Day 49. Each point represents the titer from a single animal. Horizontal lines show mean immunization group titers. The neutralizing titers of *ultra*IPV are above the protective correlate and compare favorably with the commercial vaccines. Figure 6 shows the neutralization titers of a second experiment in which rats were immunized twice with 1/5, 1/10, 1/20, and 1/40 of a standard human dose (40:8:32 D antigen units). The data from sera sampled 3 weeks after the boost show titers above the level of 1:8 ($log_2 = 3$), which is an accepted correlate of protection in humans[29,35,36].

Discussion

The development of an effective vaccine necessitates a delicate balance between mitigating adverse reactions, managing the pathogenicity of the agent during manufacturing, and ensuring vaccine efficacy. Polio was responsible for disabling 15,000–20,000 individuals annually in the US during the late 1940s. Post introduction of IPV and OPV, these figures drastically decreased to approximately 100 per year in the 1960s and to around 10 annually in the 1970s[53,58]. Since the start of the Global Polio Eradication Initiative, an estimated 2.2 million instances of deaths and 20 million cases of paralytic polio were prevented worldwide from 1988 to 2018[59].

OPV has been the workhorse throughout much of the vaccination campaigns. The use of OPV is accompanied by the risk of reversion to neuropathic forms during replication in the gut. The reversion rates have been estimated to be on the order of 1 in 125,000 birth cohorts in a Norway study, with about half in vaccinees and half in by-standers[60], 1 in 143,000 in India[61], and approximately 1 in 750,000 from a review of documented cases worldwide[62,63]. Throughout most of the seven decades of OPV use, the risk of paralytic disease from natural infection with wild-type viruses far outweighed the risks from attenuated vaccine viruses that have evolved into pathogenic strains. However, as the global burden of wild-type infection has declined, the risks of evolved viruses have eclipsed those of wild-type infections, and OPV has been replaced with IPV in most countries.

Historically, IPV has been produced by formalin inactivation of wild-type strains of PV1, PV2, and PV3 viruses. As global eradication efforts continue, the use of neuropathogenic viruses in manufacturing has become an increasingly serious biohazard and biosecurity risk. Despite manufacturers' rigorous safety measures, at least two accidental leaks into the surrounding environment have been documented[23,64,65].

In light of the risks posed by OPV reversions and IPV's wild-type strains, alternative vaccine strategies are required. New OPV products that incorporate novel OPV (nOPV) vaccine strains have been engineered to be more reversion resistant. A 2023 statement from the Global Polio Eradication Initiative reported that nOPV2, which has evolved into pathogenic strains, had been recovered from the stools of seven children with paralytic polio who were immunized with nOPV2[66]. Although this number is lower than what would have been anticipated with OPV2 (Sabin strain), any such evolved viruses are concerning.

Sabin-based IPV vaccines have been developed and approved for use in some countries, with attenuated strains used in manufacturing to reduce biohazard and biosecurity risks. However, the inactivation of PV1 Sabin with formalin has been linked to damage to a neutralizing epitope[67,68]. In addition, a study of 300 infants vaccinated with three doses of Sabin-IPV showed reduced immunity against wt-PV1 compared to infants vaccinated with conventional IPV[69]. Based on our findings of reduced antigen damage caused by UVC-inactivation compared to formalin-inactivation, the reduced immunogenicity against the wild-type PV1 component may have been caused by formalin cross-linking of amino acids within neutralization epitopes. If so, the reduction in efficacy against wt-PV1 may be avoided with the use of *ultra*IPV™.

Conflicting thoughts on the ability of IPV to lead to and maintain eradication have been published[70]. It is well-accepted that OPV stimulates high levels of mucosal immunity after replicating in the intestines. The ability of IPV to lead to and maintain eradication is not as clear, possibly due to difficulties in quantitating the level of polio-specific IgA in stool samples. However, Norway phased out OPV in favor of IPV in 1979, and since then, all reported cases of poliomyelitis have been imported[53,71]. In studies where vaccinated children were challenged with OPV, those immunized with IPV shed less fecal and nasopharyngeal virus than naïve, yet more than those initially vaccinated with OPV[53]. In addition, children immunized with three doses of cIPV had similar levels of nasopharyngeal sIgA antibodies as seen in those immunized with three doses of OPV[53,72]. Thus, it appears clear that IPVs can stimulate some level of mucosal immunity which could assist in eradication and maintaining the state of eradication.

In this report, we present data showing similar inactivation kinetics of the three polio Sabin serotypes (Fig. 1) and confirmation data showing a lack of residual infectivity after 30 s UVC treatments. We unexpectedly found that when calibrated to formalin-inactivated viruses using standard D-antigen ELISA, the UVC-inactivated viruses contained far less mass of virus protein, suggesting that the UVC-MDP inactivation method is gentler to the antigens by preserving epitopes. In addition, we found a disconnect between the D-antigen ELISA data derived from the three viruses inactivated with and without the MDP complex (Fig. 2), and the neutralization data (Fig. 5). UVC inactivation of PV1 and PV2 without MDP caused an almost complete reduction of D antigen while the reduction in neutralization stimulated by the immunogens was more modest. In contrast, the D antigen content of PV3 inactivated with or without MDP was relatively constant. The PV3 result is reproducible and not yet understood. The PV3 data may reflect the complexity of characterizing polio immunogens based on the concentration of a single epitope such as reported by the D antigen ELISA. Finally, we observed fairly consistent magnitudes of neutralization titers when assessing partial vaccine doses (Fig. 6). These results may reflect the timing of the experimental samples where immunizations occurred on Days 1 and 21 and the serum for neutralization was collected on Day 49. We hypothesize that lower doses may result in reduced neutralization titers when the sera is collected several months or years after the final immunization.

The vaccine candidate described in this report, *ultra*IPV™, incorporates at least three enhancements over previous vaccines. The inactivated candidate is produced using attenuated Sabin strains, which reduce manufacturing risks. The inactivation process takes less than a minute compared to 2–4 weeks for formalin inactivation. In addition, the increased number of doses per milligram of input virus could lead to reduced costs and increased supplies, an important feature when phasing out less expensive OPV vaccines. Moreover, the use of UVC instead of formalin inactivation may avoid damage to neutralizing epitopes, which could increase immunity to wt-PV1. In ongoing studies, we plan to develop *ultra*IPV™ through IND-enabling studies and then clinical trials. We believe that the regulatory development pathway will benefit from the long safety and efficacy record of IPV products and that we will need to demonstrate that the immunogenicity profiles (e.g., stimulated neutralizing titers) are not significantly lower and that the toxicity profiles are not significantly higher than approved conventional IPVs. We recognize that the novel inactivation process may require additional analysis to satisfy safety concerns, and the use of attenuated strains may require additional immunogenicity analyses.

Methods

Virus production

Viruses were propagated in shaking suspension culture of H1-HeLa using standard technologies[50]. Crude intracellular viruses were purified by centrifugation through a 30% w/v sucrose cushion at 120,000×*g* for 6 h, purification on Toyopearl Sulfate-650F cation exchange resin (Tosoh Biosciences) using 100 mM sodium citrate buffer at pH 6.0 with NaCl elution, and Toyopearl HW-65F size-exclusion resin (Tosoh Biosciences).

Virus infectivity titer assays

10-fold dilutions of virus samples were titrated for infectivity in MRC5 cells using standard techniques[50]. Titers were determined using the Spearman–Kärber formula (log10 50% endpoint dilution = $-(x0-d/2+d\sum ri/ni)$, where $x0$ = log10 of the reciprocal of the final dilution at which all wells are positive; d = log10 of the dilution factor; ni = number of replicate wells used; ri = number of positive wells)[73–75].

UVC inactivation of viruses

0.1–0.3 mg/ml of PV1-S, PV2-S, and PV3-S were formulated with the MDP complex consisting of 25 mM potassium phosphate buffer (pH 7.4), 2.5 mM $MnCl_2$ and 3 mM decapeptide (DP1: DEHGTAVMLK) as previously described[50]. MDP-virus samples were placed into thin-wall 0.2 ml PCR tubes, ambient air was purged with argon, and the tubes were placed onto a Model UVP UVG-54 UVC wand (Analytik Jenna US, Upland, CA) outputting 5 mW per square centimeter. UVC output was measured using a

UV512C digital light meter (General). Samples exposed for 30 s received ~120 mJ of light energy. Irradiation times are adjusted to compensate for reduced light output as the lamp ages.

Analysis of UVC-treated viruses

To determine the inactivation kinetics, samples of PV1-S, -2S, and -3S were exposed to increasing doses of UVC and then titered in 96-well plates. The wells were scored as infected or not infected based on microscopic examination of cytopathic effects (CPE). Titers were determined using the Spearman–Kärber formula as above. To determine whether inactivated viruses contained traces of residual infectivity, six-well plates of MRC-5 cells were inoculated with UVC-treated viruses and incubated for 4–5 days. The plates were examined microscopically for CPE and subjected to three freeze–thaw cycles if none was observed. A portion (~25%) of the material from the final freeze–thaw was placed on fresh MRC-5 indicator plates and incubated for another 4–5 days. The process was carried out through five passages to determine that no residual infectivity remained as per WHO guidelines[34].

D antigen ELISA

Sabin virus-specific D antigen ELISAs were used to quantify the concentration of inactivated virus and calibrate the amount of material per dose with commercially prepared IPV products[52]. The antibodies and reference standards were generously provided by Drs. Konstantin Chumakov and Diana Kouiavskaia at the FDA Office of Vaccines Research and Review. Briefly, plates were coated with polyclonal antibodies raised against PV1, 2, or 3 overnight at 4 °C. After washing and blocking, test samples and standards were incubated in the wells overnight at 4 °C. The following day, plates were washed and probed with biotinylated anti-poliovirus 1, 2, or 3 polyclonal antibodies and detected with Extravidin-HRP and 3,3',5,5'-tetramethylbenzidine substrate. Four-parameter logistic regression of the values was used to calculate unknown sample concentrations over a range of dilutions using GraphPad Prism 8.2.1. The D antigen concentrations were used to formulate standard immunization doses, each containing 40, 8, and 32 D antigen units of serotypes 1–3, respectively, in 0.5 mL volumes to correspond to commercial IPV products.

Rat immunization

Animal studies were performed under humane conditions using protocols reviewed and approved by the Institutional Animal Care and Welfare Committee at Cocalico Biologicals, Inc. (Denver, PA). All studies were performed in accordance with the guidelines of the American Veterinary Medical Association (AVMA) under the registrations of Animal Welfare Assurance number D16-00398 (A3669-01) and USDA Research License 23-B-0028. Wistar rats, a widely accepted animal model for polio vaccine analysis, between 6 and 8 weeks of age and of mixed sex were used for immunization studies. Animals were housed in a pathogen-free facility, provided food and water without restriction, and observed twice daily to assess potential health problems. Rats were immunized by intramuscular injection into the quadriceps without adjuvant on Days 1 and 21. On Day 49, the rats were anesthetized with isoflurane, bled by cardiac puncture, and then humanely euthanized by CO_2 inhalation following AMVA guildelines. Serum samples were prepared from coagulated blood to assess antiviral immune responses. No differences in health observations, weight, or behavior were detected between immunization groups before or after immunization. No adverse events were observed from the immunizations.

Virus neutralization assay

Neutralization assays were performed using standard procedures[50]. Briefly, serial two-fold dilutions of serum were incubated 1 h with 100 $CCID_{50}$ of each poliovirus in separate assays. The virus-serum mixtures were applied to six replicate wells in 96-well plates of MRC5 monolayers at room temperature. After a 1 h incubation, the plates were washed to remove unbound virus, media was added to the wells, and the plates were incubated for 4–6 days at 37 °C. Wells were scored as infected or uninfected by microscopic visualization of CPE. Neutralization titers were derived using the Spearman–Kärber method[73–76]. The titer represents the reciprocal of the highest dilution of serum that causes a 50% reduction in the number of infected wells. The neutralization titers were graphed as Log_2 values.

Statistical analysis

In order to evaluate the immunogenicity of irradiated vaccines and commercially prepared IPV vaccines, unpaired, parametric, one-tailed Student's *t*-tests were performed. Neutralizing titers stimulated by irradiated vaccine samples were compared to neutralizing titers stimulated by either IPOL^R or VeroPol^R independently to test the hypothesis that the irradiated vaccines are more immunogenic than either of the commercially prepared vaccines used in comparison. Analyses were performed using GraphPad, Prism version 8.2.1. P values are reported within the figures

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data presented in this report can be extracted from the figures for additional analyses. The authors welcome requests from qualified laboratories for additional information related to UVC-inactivation of vaccines with the MDP complex.

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Author contributions

G.J.T. designed the study. R.V.B., J.K.T., and G.J.T. propagated, purified, inactivated and titered the viruses. J.K.T. and A.V.K. performed virus neutralization analyses. S.J.D. performed D antigen quantitation studies and performed freeze–thaw experiments. J.K.T., S.J.D., D.A.M., and G.J.T. performed statistical analyses of the data. G.J.T. wrote the first draft of the manuscript, and all co-authors, including H.N.M., M.J.D., M.F.M., and T.J.W. reviewed and edited the paper. All authors have approved the submission of this manuscript.

Competing interests

G.J.T., J.K.T., R.V.B., T.J.W., S.J.D., D.A.M., and A.V.K. are employees of Biological Mimetics, Inc., which is the recipient of the NIH grant and DTRA contract that provided the majority of the funding for the project. All other authors have no competing interests.

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