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Lack of immune interference between inactivated polio vaccine and inactivated rotavirus vaccine co-administered by intramuscular injection in two animal species ☆

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ABSTRACT

A parenteral inactivated rotavirus vaccine (IRV) in development could address three problems with current live oral rotavirus vaccines (ORV): their lower efficacy in low and middle-income countries (LMICs), lingering concerns about their association with intussusception, and their requirement for a separate supply chain with large volume cold storage. Adding a new parenteral IRV to the current schedule of childhood immunizations would be more acceptable if it could be combined with another injectable vaccine such as inactivated polio vaccine (IPV). Current plans for polio eradication call for phasing out oral polio vaccine (OPV) and transitioning to IPV, initially in LMICs as a single dose booster after two doses of OPV and ultimately as a two dose schedule. Today in many LMICs, IPV is administered as a standalone vaccine, which involves a separate cold chain and is relatively costly. We therefore tested in two animal models formulations of IPV with IRV to determine whether co-administration might interfere with the immune response to each product and spare antigen dose for both vaccines. Our results demonstrate that IRV when adjuvanted with alum and administered alone or in combination with IPV did not impair the immune responses to either rotavirus or poliovirus serotypes 1, 2 and 3. Similarly, IPV when formulated and administered alone or together with IRV induced comparable levels of neutralizing antibody to poliovirus type 1, 2 and 3. Furthermore, comparable antibody titers were observed in animals vaccinated with low, middle or high dose of IPV or IRV in combination. This dose sparing and the lack of interference between IPV and IRV administered together represent another step to support the further development of this novel combination vaccine for children.

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1. Introduction

Poliovirus and rotavirus are both enteric pathogens of children that are now controlled through the use of vaccines [1–3]. Though the two viruses cause different diseases, they have some striking similarities. Live oral vaccines against poliovirus (OPV) and rotavirus (ORV) have been effective worldwide in preventing disease in children [2,4]. However, both vaccines are associated with rare but severe adverse events, such as vaccine-associated paralytic polio (VAPP) or circulating vaccine-derived polioviruses (cVDPV) for OPV and intussusception following ORV in vaccinated children [5–7]. Also, both oral vaccines are more immunogenic and effective among children in developed and middle-income countries than in low-income countries. The mechanisms for this lower effectiveness are not understood but may be related to environmental enteropathy or mixed infections in the gut, higher levels of maternal antibody transferred to the infant through the placenta or breastmilk, or deficiency in micronutrients among children in developing countries [8,9]. In the absence of full understanding of this problem, its solution rests in part upon finding alternative vaccines that are not administered by mouth.

☆ The finding and conclusions in this report are those of the authors and do not necessarily represent the official positions of Centers for Disease Control and Prevention.

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Despite the introduction of rotavirus vaccines more than a decade ago, rotavirus remains the leading cause of severe diarrhea among young children worldwide, even in those LMICs where ORVs have been introduced into national programs of immunization [10]. To improve the effectiveness and safety of current rotavirus vaccination programs, parenteral rotavirus vaccine candidates derived from inactivated whole virus, subunit recombinant proteins or protein fragments are all being developed [10,11]. An inactivated rotavirus vaccine (IRV) when formulated with alum gel and administered by intramuscular injection has proven to be highly immunogenic in mice and guinea pigs and protective against an oral challenge with a virulent human strain in piglets [12–14].

Inactivated polio vaccine (IPV), first licensed in 1950s, has been highly successful in preventing clinical poliomyelitis in children and has led to a dramatic reduction in infantile paralysis [15]. However, OPV replaced IPV globally because of its lower cost, ability to provide herd protection, and ease of administration orally. New understanding of the ability of OPV to, on rare occasions, revert to virulence and cause VAPP has led to a reassessment of the critical role that IPV will play in the global polio eradication endgame. Several countries in northern Europe eradicated polio early on through exclusive use of IPV. In LIMCs, administration of an IPV booster dose can effectively enhance intestinal immunity in children who responded poorly to multiple doses of OPV and prevent the emergence of vaccine-derived polio. Today, IPV has been integrated into the universal immunization program in India and many other countries and has become a critical component of the polio endgame worldwide [16].

The World Health Organization recently recommended a two-dose IPV immunization regimen at 10–14 weeks and 9 months of age when OPV is withdrawn globally to sustain polio eradication in the coming years [17]. Since IPV currently is administered alone in most developing countries where ORVs are less effective in the first year and their protective immunity is further waned in the second year [18], we see a strong scientific and programmatic rationale for developing a combination vaccine composed of IPV and IRV as a booster vaccine to improve the efficacy and safety against both poliovirus and rotavirus in LMICs and beyond. In the present study, we examined whether vaccinating two model animal species, guinea pigs and rats, with a combined IPV-IRV product might inhibit the immune response to either of these vaccines. A lack of interference would be important to encourage the further development of this approach.

2. Methods

2.1. IRV and IPV preparations

Two IRV pilot lots, one prepared by The Serum Institute of India Pvt. Ltd. (SIIPL) and the other by Meridian Life Science (MLS) and formulated at CDC were tested. For the SIIPL lot, VERO cells in roller bottles were infected with vaccine strain CDC-9 (G1P[8]) [19] incubated at 37 °C for 72 h, and the virus was harvested by freezing and thawing at −70 °C twice. The harvested virus was clarified using a 30 μm + 2 μm filter assembly and treated with benzonase (EMD Millipore, Darmstadt, Germany) to break down any host cell DNA. Tangential flow filtration was used to remove host cell proteins, degraded DNA, and residual benzonase, and the virus was concentrated. The virus was then diafiltered to bring into Hanks Balanced Salt Solution supplemented with 10% sorbitol, the final diluent for the vaccine. Finally, the virus was heat inactivated at 60 °C for 5 h and complete inactivation was confirmed by the absence of growth following three serial passages in MA104 cells. Samples of inactivated virus were removed, and the aliquots of inactivated virus were stored at −20 °C until use. Sterility was checked for bacteria and fungi at each level during upstream and downstream processing of the virus. Total antigen (protein) content was determined by the Bradford method (Sigma Aldrich, St. Louis, MO). The CDC IRV lot was similarly prepared except that two column chromatography steps were included to further purify the virus in the downstream. All release tests for sterility, residual host DNA and HCP, and vaccine potency were conducted and met the relevant regulatory requirements.

SIIPL used Salk inactivated polio antigen bulk and Sabin live monovalent bulk of type 1, 2 and 3 from commercial manufacturers. Sabin live viruses were inactivated using formalin (0.025%) for 13 days at SIIPL. CDC used a commercial Salk IPV.

2.2. Immunogenicity testing in animals

SIIPL prepared and tested four formulations of IRV-IPV combination vaccine in guinea pigs. Each formulation had 10 μg of IRV combined with either a full or fractional dose Salk or Sabin IPV (Table 1). All individual antigens were adsorbed on aluminum hydroxide to bring the total aluminum hydroxide per dose of 500 μl to <1 mg. Formulations 1 and 3 were each tested in four guinea pigs whereas formulations 2 and 4 were each tested in two guinea pigs. Two guinea pigs received 10 μg (total protein) of the live human rotavirus CDC-9 ($10^{5.5}$ fluorescent forming units) adsorbed on aluminum hydroxide as a positive control and two guinea pigs were uninoculated as negative controls. Each guinea pig was immunized at days 0, 14 and 28. Serum samples were collected on days 0 and 42 to evaluate poliovirus- or rotavirus-specific antibody response before and after vaccination.

CDC prepared IRV and IRV plus Salk IPV combination formulations with 600 μg of aluminum hydroxide per dose (Table 2). These vaccine formulations were tested in pathogen-free female Wistar rats (6–8 weeks old) (Charles River Laboratories, Wilmington, MA) housed in the CDC animal facility. Before the administration, rats were anesthetized with 3–5% isoflurane for induction in a chamber and approximately 1–2% for maintenance, through a nose cone, and were pre-bled from the submandibular vein with an animal lancet (Medipoint International, Mineola, NY). Rats in groups of 4 or 5 received 3 doses of standalone IRV or IPV, or IRV-IPV combination vaccine at 100%, 50% and 25% human dose on days 0, 21, and 42. Serum was collected before each vaccination and at day 63,

Table 1
Type and compositions of four IRV-IPV combination vaccine formulations at SIIPL.

Formulation	IPV	IPV type and antigen dose			IRV antigen dose
		IPV-1	IPV-2	IPV-3	
1	Salk	8 DU	2 DU	5 DU	10 μg
2	Salk	5 DU	2 DU	5 DU	10 μg
3	Sabin	5 DU	16 DU	10 DU	10 μg
4	Sabin	2.5 DU	8 DU	5 DU	10 μg

DU = D antigen unit.

Table 2
Compositions of IRV, IPV and IRV-IPV combination vaccine formulations at CDC.

Vaccine dosage	Vaccine	IPV type and antigen dose			IRV antigen dose
		IPV-1	IPV-2	IPV-3	
100%	IRV	–	–	–	10 μg
	IRV-IPV	40 DU	8 DU	32 DU	10 μg
	IPV	40 DU	8 DU	32 DU	–
25%	IRV	–	–	–	2.5 μg
	IRV-IPV	10 DU	2 DU	8 DU	2.5 μg
	IPV	10 DU	2 DU	8 DU	–
50%	IRV-IPV	20 DU	4 DU	16 DU	5.0 μg

Vaccine dosage refers to established human dose for commercial IPV. For IRV, we defined 10, 5 and 2.5 μg as 100%, 50% and 25% dose, respectively, in this study. DU = D antigen unit.

3 weeks after the final vaccine dose. Control animals received placebo (600 μg of aluminum hydroxide) in the same manner. All animal experiments were approved by the Institutional Animal Ethical Committee (IAEC) of SIIPL and the institutional animal care and use committee (IACUC) of CDC and conducted in accordance with the ethical guidelines for animal experiments and safety guidelines.

Rotavirus-specific IgG in animal sera was measured by enzyme immunoassay [20]. Antibody titer in a serum specimen was defined as the reciprocal of the highest dilution that gave a geometric mean OD greater than 3 standard deviations above the mean OD of the negative-serum wells. Rotavirus neutralizing antibody was measured against human virus Wa, a homotypic G1P[8] strain, and MW333, a heterotypic G8P[4] strain, with a microneu-

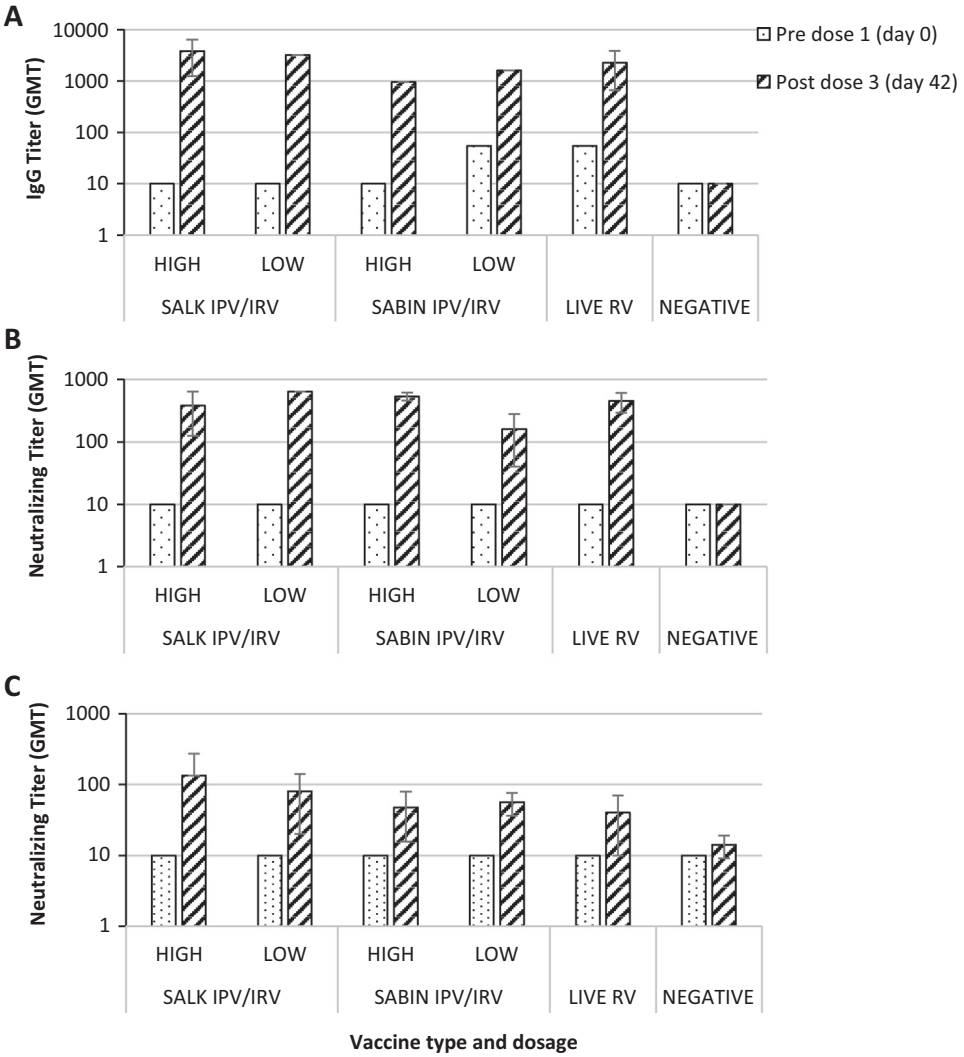

Fig. 1. IRV (SIIPL) in IRV-IPV (Salk and Sabin) combination vaccine and live rotavirus vaccine induce comparable titers of rotavirus-specific IgG and neutralizing activity in sera of guinea pigs. Animal serum was collected before the first vaccine dose (day 0) and after the third dose (day 42), and was tested for rotavirus IgG (A), neutralizing activity against the homotypic strain Wa (B), and the heterotypic strain MW333 (C). Control guinea pigs received live rotavirus CDC-9 or placebo. Serum specimens were tested for IgG at an initial dilution of 1:100 and if negative, a value of 20 was used for determining geometric mean titers (GMT) and illustration. Neutralizing antibody was tested at an initial dilution of 1:20. Error bars represent one standard error of the mean.

tralization assay [14]. Neutralizing antibody titer was defined as the reciprocal of the highest dilution that gave a greater than 70% reduction in the absorbance value compared with that in virus-only controls. Poliovirus neutralizing antibody was measured against Sabin virus types 1, 2 and 3 by microneutralization assays [21]. Statistic differences in antibody titer between groups were assessed using two-way ANOVA and Tukey's multiple comparison test with significance being $p \leq 0.05$.

3. Results

We first compared the immunogenicity of the IRV-IPV combination vaccine and a live human rotavirus vaccine with the same amount of viral protein content, both formulated with aluminum hydroxide and administered IM, in guinea pigs at SIIPL (Fig. 1). IRV formulated with high-dose Salk (formulation 1) or Sabin (formulation 3), or low-dose Salk (formulation 2) or Sabin (formulation 4) IPV in the combination vaccine induced elevated geometric mean titers (GMT) of rotavirus-specific IgG (16- to 128-fold) and neutralizing activity against both the homotypic Wa (8- to 128-fold) and heterotypic MW333 (2- to 64-fold) strains. These titers were comparable to those induced by the live virus. Unimmunized animals remained negative for rotavirus antibody throughout the study.

IPV formulations with IRV in the combination vaccine all induced protective titers (≥1:8) of neutralizing antibody to polio Sabin types 1, 2 and 3 (Fig. 2). Salk and Sabin IPV type 2 in the combination vaccine was the most immunogenic and both vaccines induced similar GMTs to Sabin type 2 at the high and low doses. As expected, Salk IPV at low dose induced lower GMTs to Sabin types 1 and 3 than Sabin IPV. None of the control animals had detectable neutralizing antibody to polioviruses.

We then tested the CDC preparation of IRV combined with a commercial Salk IPV that was administered in three formulations – full-strength (100% or 10 μg), half-strength (50% or 5 μg) and

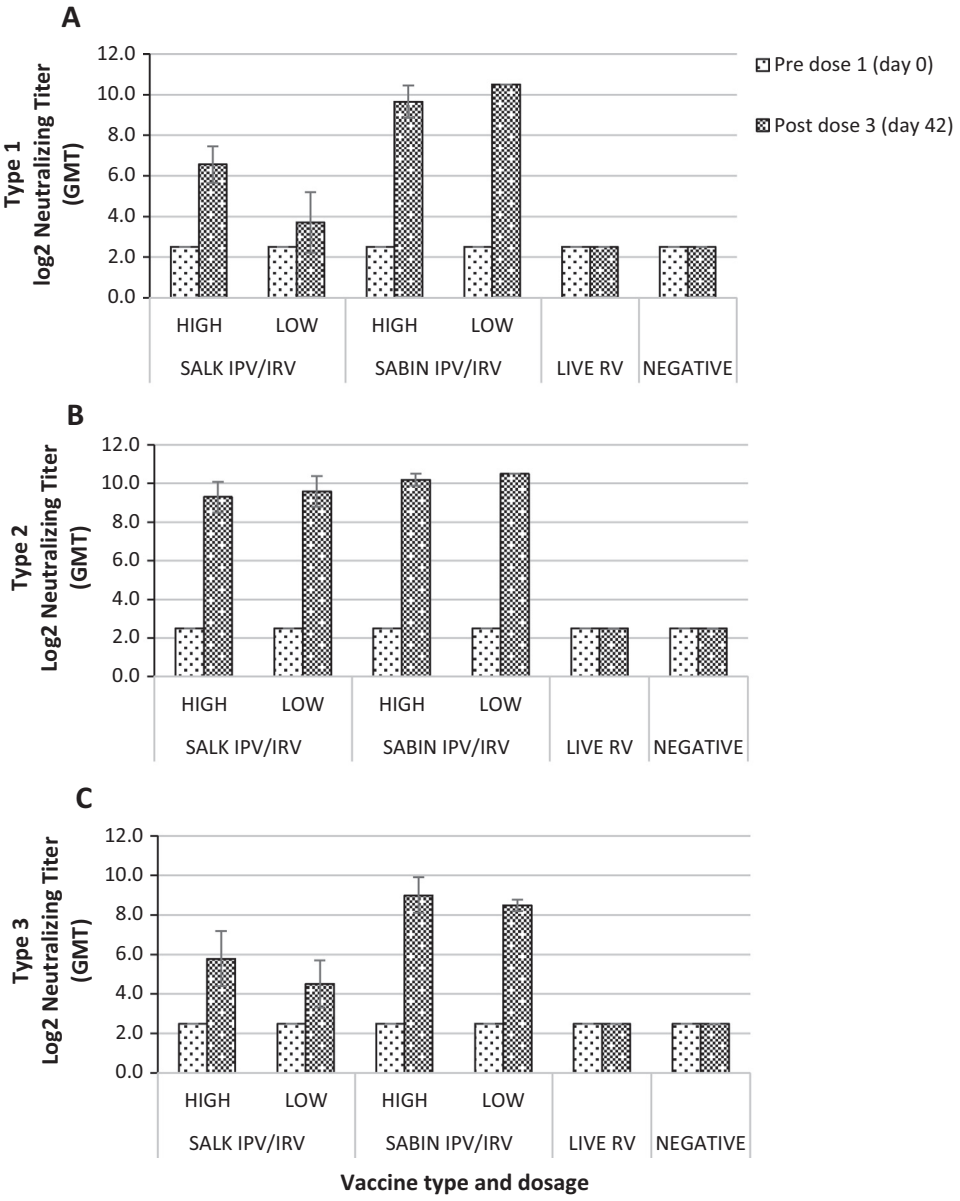

Fig. 2. Salk IPV and Sabin IPV in IRV-IPV combination vaccine induce protective titers of neutralizing antibody to poliovirus in sera of guinea pigs. Animal serum was collected before the first vaccine dose (day 0) and after the third vaccination dose (day 42), and was tested for neutralizing antibody against polio Sabin type 1 (A), type 2 (B) and type 3 (C). Control guinea pigs received live rotavirus CDC-9 or placebo. Antibody titers are expressed as GMT for each group (n = 2 or 4). Error bars represent one standard error of the mean.

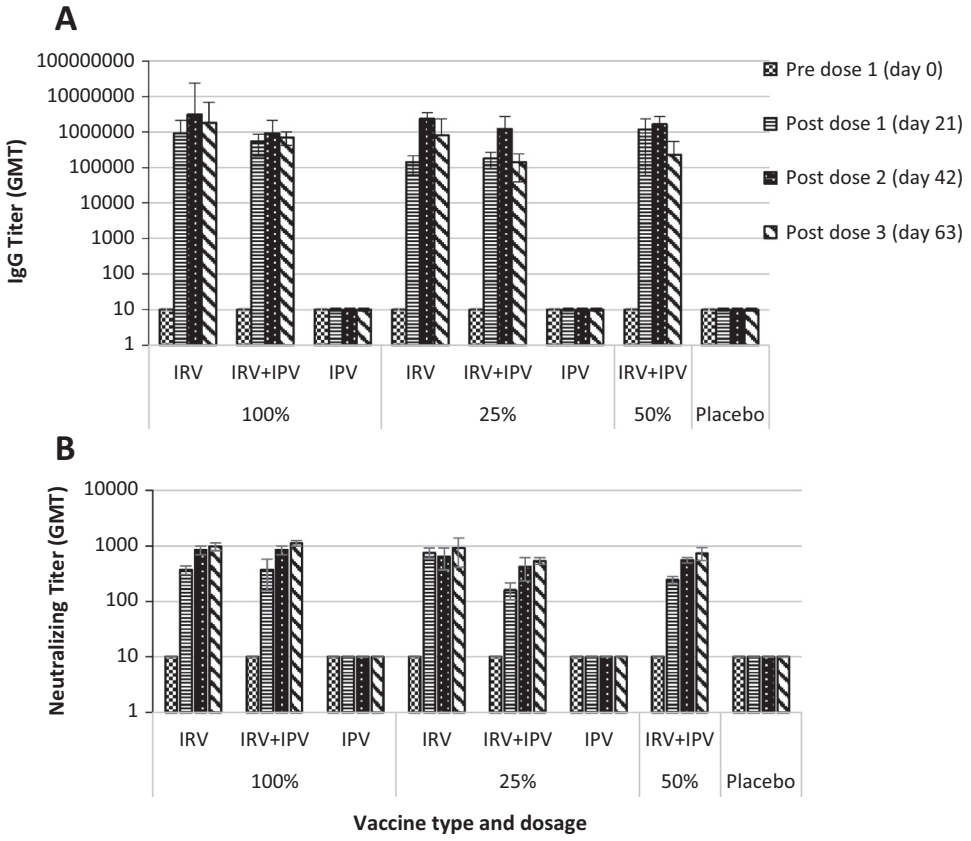

Fig. 3. IRV (CDC) alone and in the IRV-IPV (Salk) combination vaccine induce comparable titers of rotavirus-specific IgG and neutralizing activity in sera of rats. Animal serum was collected before the first, second and third vaccine dose (day 0, 21, 42) and after the third vaccination dose (day 63), and tested for rotavirus IgG (A) and neutralizing activity (B) against the homotypic strain Wa. Control rats received placebo. Serum specimens were tested for IgG at an initial dilution of 1:100 and if negative, a value of 20 was assigned for determining GMT and illustration. Neutralizing antibody was tested at an initial dilution of 1:20. Error bars represent one standard error of the mean. Post-dose 3 antibody titers to rotavirus among full- and fractional-strength formulations of standalone IRV or combined IRV-IPV were not statistically significant ($p > 0.05$).

quarter-strength (25% or 2.5 μg) in rats. After three inoculations, IPV at 100% and 25% human doses did not significantly affect either the IgG or the homotypic neutralizing response to rotavirus in the combination vaccine when compared with IRV administered alone (Fig. 3). No rotavirus antibody was detected in animals that received IPV or placebo. Similarly, at full and quarter doses of the combination IPV-IRV vaccine, the neutralizing responses to the three Sabin polio serotypes did not differ significantly from the responses to IPV when administered alone (Fig. 4). No polio antibody was detected in animals that received only IRV or placebo. We observed lower antibody titers in animals that received one or two doses of IPV, in particular Salk 3. A third injection of IPV alone or in combination, even at the 25% dose, was able to enhance neutralizing antibody titers to levels similar to those from full doses. Of note, similar antibody titers to rotavirus and polio were observed in rats that received IRV-IPV combination vaccines at full or fractional (50% or 25%) doses (Figs. 3 and 4). IRV or IPV at the 50% dose when administered alone was not tested.

4. Discussion

Clinical studies of oral vaccines in infants have demonstrated that OPV interferes with ORV when the two vaccines were administered together and noted that this interference was particularly apparent after the first or second ORV dose [21,22]. Our findings for parenteral vaccines in animal models, guinea pigs and rats in which IPV is usually more immunogenic, are quite different. We found that the antibody titers to rotavirus and poliovirus were comparable in animals that received IRV or IPV alone, or in the IRV-IPV combination, indicating no interference in immune response. We further observed similar antibody titers in animals that received combination vaccines in different formulations with full or fractional doses and thus demonstrated apparent dose sparing for both IPV and IRV. These results were robust for two different animal species and with IPV in formulations from different sources. In addition, we found no difference in the immunogenicity of the live and inactivated rotavirus preparations, indicating that antigenic epitopes in IRV were not altered after heat inactivation. We demonstrated strong immunogenicity of the two IRV pilot lots prepared at SIIPL and CDC, supporting that either lot is suitable for use in an IPV-IRV combination vaccine. Clearly, animal data have their limitations and this study with small numbers of animals cannot predict the outcome in infants. Nonetheless, it suggests that the antigens for IPV and IRV when formulated with alum did not interfere in the models tested.

The immunogenicity and efficacy of ORVs are lower in the first year of life and further decline in the second year in many LMICs [18]. A booster dose of Rotarix at 9 month of age has shown not to interfere with measles-rubella vaccine when given simultaneously to children, but only slightly improved ORV immunogenicity in Bangladesh [22]. This small improvement in immunogenicity has raised the question about the value of a booster dose of ORV in developing countries. On the other hand, an IPV booster dose both increased polio neutralizing antibody titers in serum and reduced poliovirus shedding in stool of OPV-immunized children

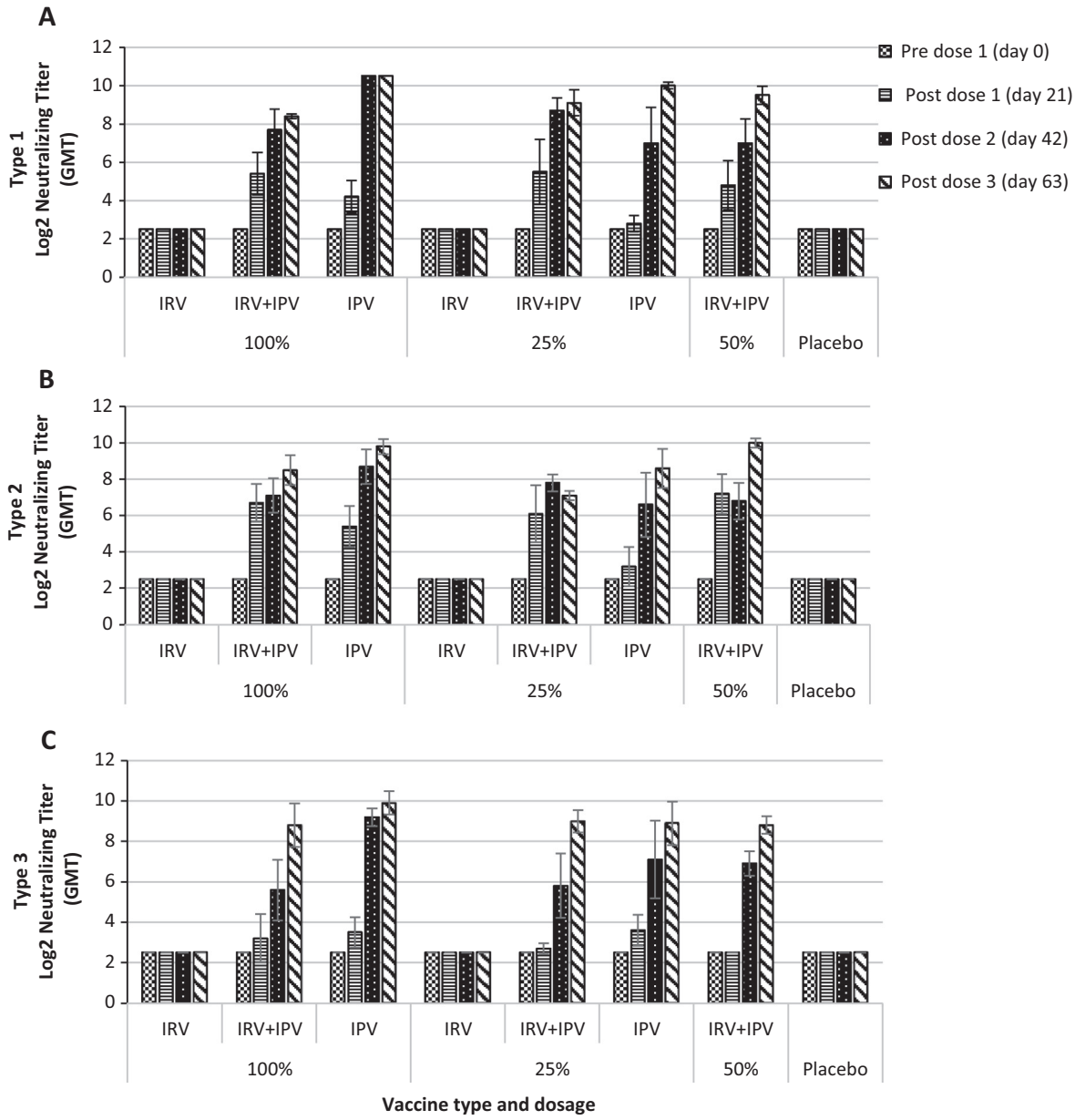

Fig. 4. Salk IPV alone and in an IRV-IPV combination vaccine induce comparable titers of polio-specific neutralizing antibody in sera of rats. Animal serum was collected before the first, second and third vaccine dose (day 0, 21, 42) and after the third vaccination dose (day 63), and was tested for neutralizing antibody against polio Sabin type 1 (A), type 2 (B) and type 3 (C) as described in the text. Control rats received placebo. Antibody titers are expressed as GMT for each group (n = 4 or 5). Error bars represent one standard error of the mean. Post-dose 3 antibody titers to polio types 1, 2 and 3 among full- and fractional-strength formulations of standalone IPV or combined IPV-IRV were not statistically significant ($p > 0.05$).

in India [16]. We foresee that an IRV-IPV combination vaccine administered at 10–14 weeks and at 9 months, together with other pediatric vaccines for routine immunization, may eliminate the intussusception risk associated with ORVs, enhance protection against rotavirus diarrhea, and help ensure polio eradication. Alternatively, a single dose of the combination vaccine at 10–14 weeks could serve as an effective booster for both OPV and ORV immunizations. The ultimate decision on this combination strategy will be determined by the speed with which IRV can be developed, the future rollout and uptake of IPV, and the willingness of LMICs to embrace this approach. As IRV-IPV combination vaccine is further developed, similar studies will be needed to demonstrate that this combination vaccine would similarly not interfere with the immune response to other potential candidate vaccines considered as combination partners.

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Conflicts of interest

Yuhuan Wang, Roger Glass and Baoming Jiang hold patents through CDC for their vaccine work. Jagdish Zade, SS Pisal, and

Rajeev M Dhere are employees of SIIPL. Rajeev M Dhere, SS Pisal, Jagdish Zade, Baoming Jiang and Roger Glass are inventors to a patent application with application number PCT/IB2017/055100 entitled "Novel Multivalent Vaccine Composition" filed jointly by SIIPL, India and CDC, USA. Authors have no other conflicts of interests.

References

[1] Tate JE, Burton AH, Boschi-Pinto C, Parashar UD. World Health Organization-coordinated global rotavirus surveillance N. global, regional, and national estimates of rotavirus mortality in children <5 years of age, 2000–2013. Clin Infect Dis 2016;62(Suppl 2):S96–S105.

[2] Sutter RW, Kew OM, Cochi SL, Aylward RB. Poliovirus vaccine – Live. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. Elsevier; 2013. p. 598–645.

[3] Robertson SE, Mayans MV, El-Husseiny A, Clemens JD, Ivanoff B. The WHO Vaccine Trial Registry. Vaccine 2001;20:31–41.

[4] Burnett E, Jonesteller CL, Tate JE, Yen C, Parashar UD. Global impact of rotavirus vaccination on childhood hospitalizations and mortality from diarrhea. J Infect Dis 2017;215:1666–72.

[5] John TJ. Problems with oral poliovaccine in India. Indian Pediatr 1972;9:252–6.

[6] Kew OM, Sutter RW, de Gourville EM, Dowdle WR, Pallansch MA. Vaccine-derived polioviruses and the endgame strategy for global polio eradication. Annu Rev Microbiol 2005;59:587–635.

[7] Patel MM, Haber P, Baggs J, Zuber P, Bines JE, Parashar UD. Intussusception and rotavirus vaccination: a review of the available evidence. Expert Rev Vaccines 2009;8:1555–64.

[8] Patriarca PA, Wright PF, John TJ. Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. Rev Infect Dis 1991;13:926–39.

[9] Velasquez DE, Parashar U, Jiang B. Decreased performance of live attenuated, oral rotavirus vaccines in low-income settings: causes and contributing factors. Expert Rev Vaccines 2018;17:145–61.

[10] Glass RI, Jiang B, Parashar U. The future control of rotavirus disease: can live oral vaccines alone solve the rotavirus problem? Vaccine 2018;36:2233–6.

[11] Jiang B, Gentsch JR, Glass RI. Inactivated rotavirus vaccines: a priority for accelerated vaccine development. Vaccine 2008;26:6754–8.

[12] Wang Y, Azevedo M, Saif LJ, Gentsch JR, Glass RI, Jiang B. Inactivated rotavirus vaccine induces protective immunity in gnotobiotic piglets. Vaccine 2010;28:5432–6.

[13] Resch TK, Wang Y, Moon SS, Joyce J, Li S, Prausnitz M, et al. Inactivated rotavirus vaccine by parenteral administration induces mucosal immunity in mice. Sci Rep 2018;8:561.

[14] Jiang B, Wang Y, Glass RI. Does a monovalent inactivated human rotavirus vaccine induce heterotypic immunity? Evidence from animal studies. Hum Vaccin Immunother 2013;9:1634–7.

[15] Nathanson N, Kew OM. From emergence to eradication: the epidemiology of poliomyelitis deconstructed. Am J Epidemiol 2010;172:1213–29.

[16] John J, Giri S, Karthikeyan AS, Lata D, Jeyapaul S, Rajan AK, et al. The duration of intestinal immunity after an inactivated poliovirus vaccine booster dose in children immunized with oral vaccine: a randomized controlled trial. J Infect Dis 2017;215:529–36.

[17] Meeting of the Strategic Advisory Group of Experts on immunization, April 2017 – conclusions and recommendations. Wkly Epidemiol Rec. 2017;92:301–20.

[18] Patel M, Glass RI, Jiang B, Santosham M, Lopman B, Parashar U. A systematic review of anti-rotavirus serum IgA antibody titer as a potential correlate of rotavirus vaccine efficacy. J Infect Dis 2013;208:284–94.

[19] Esona MD, Foytich K, Wang Y, Shin G, Wei G, Gentsch JR, et al. Molecular characterization of human rotavirus vaccine strain CDC-9 during sequential passages in Vero cells. Hum Vaccin 2010;6.

[20] Jiang B, Wang Y, Saluzzo JF, Bargeron K, Frachette MJ, Glass RI. Immunogenicity of a thermally inactivated rotavirus vaccine in mice. Hum Vaccin 2008;4:143–7.

[21] Weldon WC, Oberste MS, Pallansch MA. Standardized methods for detection of poliovirus antibodies. Methods Mol Biol 2016;1387:145–76.

[22] Zaman K, Fleming JA, Victor JC, Yunus M, Bari TI, Azim T, et al. Noninterference of rotavirus vaccine with measles-rubella vaccine at 9 months of age and improvements in antirotavirus immunity: a randomized trial. J Infect Dis 2016;213:1686–93.