

Intradermal Inactivated Poliovirus Vaccine: A Preclinical Dose-Finding Study

Diana Kouivaskaia,¹ Olga Mirochnitchenko,¹ Eugenia Dragunsky,¹ Efrat Kochba,² Yotam Levin,² Stephanie Troy,³ and Konstantin Chumakov¹

¹Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, Maryland; ²NanoPass Technologies, Nes Ziona, Israel; and ³Eastern Virginia Medical School, Norfolk

Intradermal delivery of vaccines has been shown to result in dose sparing. We tested the ability of fractional doses of inactivated poliovirus vaccine (IPV) delivered intradermally to induce levels of serum poliovirus-neutralizing antibodies similar to immunization through the intramuscular route. Immunogenicity of fractional doses of IPV was studied by comparing intramuscular and intradermal immunization of Wistar rats using NanoPass MicronJet600 microneedles. Intradermal delivery of partial vaccine doses induced antibodies at titers comparable to those after immunization with full human dose delivered intramuscularly. The results suggest that intradermal delivery of IPV may lead to dose-sparing effect and reduction of the vaccination cost.

Keywords. inactivated poliovirus vaccine; intradermal; immunogenicity; dose sparing.

Since its launch in 1988, the Global Polio Eradication Initiative (GPEI) has reduced the global incidence of paralytic poliomyelitis by >99% and the number of countries with endemic polio to just 3—Afghanistan, Nigeria, and Pakistan [1]. However, in May 2014, in response to recent outbreaks in Syria, Cameroon, and the horn of Africa, the World Health Organization (WHO) declared the international spread of polio a “public health emergency of international concern.” The primary weapon used in the global campaign has been oral polio vaccine (OPV), a live attenuated vaccine that is inexpensive, is easy to administer, and promotes herd immunity. By its nature,

attenuated RNA virus used in manufacture of OPV is unstable and can mutate into pathogenic vaccine-derived polioviruses (VDPVs) that can cause outbreaks of paralytic disease [2, 3]. The attack rate and severity of disease associated with VDPVs are similar to those caused by wild poliovirus [4]. In addition, attenuated strains of poliovirus can establish persistent infection in individuals with some forms of immunodeficiency [3]. Therefore, complete polio eradication can be achieved only after OPV use is discontinued.

In an important step, the 2012 meeting of the WHO Strategic Advisory Group of Experts on Immunization (SAGE) recommended the withdrawal of the type 2 component of OPV. The transition from the routine use of trivalent OPV to the exclusive use of bivalent vaccine will be made after all outbreaks caused by circulating VDPV type 2 will be stopped, and after introduction of at least 1 dose of the inactivated polio vaccine (IPV) [5] to maintain immunity to all 3 serotypes of poliovirus. Although IPV is believed to produce inferior intestinal immunity compared with OPV [6], it is effective at preventing poliomyelitis, and has been successfully used to eradicate polio and/or to maintain the absence of paralytic poliomyelitis in the developed world. In April 2014, SAGE supported a global introduction of IPV prior to OPV withdrawal in all countries, as part of the end-game strategic plan [7].

IPV's main drawback is its cost; it is approximately 20 times more expensive than OPV, limiting its use in resource-limited countries [8]. One way to decrease the cost of IPV immunizations is to increase the immunogenicity of IPV that could enable the reduction of the necessary vaccine dose. The dose-sparing effect could be achieved by using the intradermal rather than intramuscular route of administration. Because of the high density of dendritic cells in the skin, it has been possible to reduce the dose of a number of vaccines when they are administered intradermally, such as the rabies and influenza vaccines [8]. Recent studies that have compared intradermal vaccination with 20% of a standard dose of IPV to full-dose IPV given intramuscularly produced mixed results on seroconversion but showed uniformly lower antibody titers in the intradermal group [8–10]. It is unclear whether these inferior results were because a fractional dose >20% of the standard intramuscular dose is needed for IPV given intradermally, or whether needleless intradermal delivery devices used in these studies imprecisely targeted the dermis (eg, occasionally delivered too deep or with partial leaks). To help answer these questions, we conducted a preclinical study in Wistar rats comparing the serologic response to a 3-dose schedule of 5%, 10%, 20%, or 40% IPV given either intradermally or intramuscularly with 100% IPV

Received 20 August 2014; accepted 30 October 2014; electronically published 12 November 2014.

Correspondence: Konstantin Chumakov, PhD, Center for Biologics Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Ave, Bldg 52, Rm 1128, Silver Spring, MD 20993 (konstantin.chumakov@fda.hhs.gov).

The Journal of Infectious Diseases® 2015;211:1447–50

Published by Oxford University Press on behalf of the Infectious Diseases Society of America 2014.

This work is written by (a) US Government employee(s) and is in the public domain in the US.

DOI: 10.1093/infdis/jiu624

given intramuscularly. The intradermal injections were performed using the NanoPass MicronJet600, which is a Food and Drug Administration (FDA)-registered, single-use device consisting of an array of 3 short microneedles that can be used with any standard syringe to deliver any liquid substance directly into the skin. MicronJet600 is 0.6 mm long and as such, enables consistent and shallow delivery into the skin, regardless of skin site, sex, body mass index, and age [11]. Previous clinical studies using the device, which were conducted mostly with influenza vaccines, have shown both significant dose sparing as well as superior immunogenicity compared with full-dose influenza vaccine, despite using one-fifth and three-fifths of the dose [12, 13].

MATERIALS AND METHODS

Immunization

Animal procedures were approved by Center for Biologics Evaluation and Research/FDA Institutional Animal Care Committee and performed in accordance with the Guide for the Care and Use of Laboratory Animals [14]. Female Wistar rats (Charles River Laboratories, Wilmington, Massachusetts) were received at the age of approximately 7–8 weeks (weight 150 g), and used in the experiments 1 week later. Each immunization dose and route was tested on a group of 10 rats, which were immunized on days 0, 21, and 35. One group (10% intradermal) consisted of 9 rats for the reason that 1 animal did not recover from anesthesia. Rats were immunized with various doses (5%–100% of 1 human dose) of IPV (IPOL, Sanofi Pasteur, Lyon, France) through intramuscular or intradermal route. Intramuscular immunizations were done by injecting vaccine in a volume of 0.1–0.25 mL into both thighs using a syringe with 25 G needle. For 5% dose, the vaccine was diluted with medium 199 (Invitrogen). For intradermal immunization, animals were anesthetized with a ketamine/xylazine mixture injected intraperitoneally. Skin area on both thighs was shaved and cleaned with alcohol. Vaccine was injected using MicronJet 600 microneedle devices (NanoPass Technologies Ltd, Nes Ziona, Israel) in a maximum volume of 0.05 mL per injection site (0.05–0.2 mL total). Successful intradermal injection was confirmed by visual appearance of a characteristic bleb. Rats immunized intradermally were observed for signs of inflammation at the site of injection. The animals were weighed throughout the study. Blood samples from immunized animals were collected on days 21 and 35 just before immunization as well as on days 49 and 77 to determine poliovirus-neutralizing antibodies.

Microneutralization Test

Poliovirus neutralizing antibody titers were determined in microneutralization test according to the WHO protocol [15]. In brief, the serum samples were heat inactivated, and 2-fold serial dilutions of the samples in triplicates were incubated with 100

tissue culture infectious doses (TCID₅₀) of respective wild poliovirus strain (type 1 Mahoney, type 2 MEF-1, and type 3 Saukett) in equal volumes for 3 hours at 36°C. HEp-2C cells (1–2 × 10⁴ per well) were added at the end of the incubation. The plates were incubated for at 36°C, and cytopathic effect was recorded after 10 days of incubation. Neutralizing titers were calculated using the Kärber formula and expressed as reciprocal of the highest dilution of serum that protects 50% of cell cultures.

Statistical Analysis

The *t* test was performed on log₂-transformed titers using Microsoft Excel spreadsheets. Seroconversion rates were compared using Fisher exact test.

RESULTS

The goal of the study was to compare postimmunization titers of poliovirus-neutralizing antibodies in groups of animals that received IPV by 2 different routes of administration. There was no weight loss after immunizations by either route, and animals demonstrated equal weight gain later (data not shown). Monitoring of the signs of local reactions in rats immunized intradermally revealed a characteristic bleb and skin redness at the site of injection immediately after injection. No other reactions were noted in any of the animals.

The serum from animals was tested by microneutralization assay. Seroconversion rates, defined as the percentage of rats that developed neutralizing antibodies detectable in dilutions ≥1:8 (considered to be protective against all 3 serotypes), are shown in Table 1 (data shown for days 21 and 35). Geometric mean titers of neutralizing antibodies are presented in Table 2. Immunizations with fractional doses of IPV were less effective than with full dose and resulted in seroconversion in a lower percentage of animals. All rats seroconverted in response to 2 immunizations with 40% or 20% of 1 human dose regardless of injection route, with the exception of 1 rat in the 40% intramuscular group for serotype 1. In groups that received 10% of full human dose, 80%–100% of the animals seroconverted. Administration of 5% of 1 human dose led to seroconversion in slightly higher numbers of animals after 2 intradermal immunizations (Table 1), although this difference was not statistically significant. To estimate the levels of neutralizing antibodies at later time points after immunizations, we determined the neutralizing titers at 6 weeks after the last immunization (77 days after the first immunization; Supplementary Figure 1, Supplementary Table 1). At days 49 and 77 (data not shown), the vast majority of rats (90%–100%) had protective levels of neutralizing antibodies regardless of the route of injection or poliovirus type.

After 2 immunizations with IPV, the rats that received 40% of the full dose intradermally vs intramuscularly had significantly higher titers for serotype 1, but no other differences reached

Table 1. Vaccine Response Rate^a

Route of Immunization	Percentage of Full Human Dose	Type 1		Type 2		Type 3	
		Day 21	Day 35	Day 21	Day 35	Day 21	Day 35
Intramuscular	5%	10	30	30	60	10	40
	10%	0	100	90	100	10	90
	20%	40	100	100	100	60	100
	40%	60	90	100	100	50	100
	100%	80	100	100	100	100	100
Intradermal	5%	0	70	60	80	0	70
	10% ^b	11	89	78	78	0	100
	20%	0	100	100	100	70	100
	40%	40	100	100	100	50	100

^a Vaccine response rate was defined as percentage of animals with poliovirus neutralizing titer ≥ 8 .

^b Group consisted of 9 animals.

significance. However, by 2 weeks after the third IPV dose and continuing through at least 6 weeks after the third IPV dose, intradermal vaccination at 20% or 40% of the full dose produced higher antibody titers for all serotypes compared with the equivalent dose given intramuscularly. This observation reached significance ($P < .05$) for the 20% dose for serotypes 2 and 3 both 2 and 6 weeks after the third immunization, and for the 40% dose for serotypes 1 and 2 both 2 and 6 weeks after the third immunization, and for serotype 3 two weeks after the third immunization (Table 2 and [Supplementary Table 1](#)).

DISCUSSION

Here we report the results of our laboratory study in which Wistar rats (in groups of 10) received 3 immunizations each of a fractional IPV dose (5%, 10%, 20%, or 40% of the standard human dose) given either intradermally or intramuscularly, vs the full dose of intramuscular IPV. We found that both the 20% and 40% fractional intradermal dose of IPV as compared to the

100% intramuscular dose of IPV resulted in noninferior seroconversion rates (as measured by neutralizing antibody titers of $\geq 1:8$) for all 3 serotypes by 14 days after the second vaccine dose, which satisfied the primary aim of the study. Furthermore, we noted that the second booster dose seemed to generate more robust responses in the intradermal vs the intramuscular group. Finally, the safety profile, as measured by weight gain in the rats, was roughly equivalent for the intradermal vs the intramuscular routes.

The relatively consistent dose response for the intradermal administration of IPV in our study has implications for additional human studies. The results of our study suggest that intradermal administration of a fractional dose $>20\%$ might produce equivalent antibody titers as 100% intramuscular doses, if the dose-response curve we saw in rats proves to be similar in humans. We are currently conducting a trial administering a booster dose of 40% intradermal, 20% intradermal, 40% intramuscular, and 100% intramuscular IPV in humans to test this hypothesis.

Table 2. Neutralizing Titers in Groups of Animals Immunized With Various Doses of Inactivated Polio Vaccine

Route of Immunization	Percentage of Full Human Dose	Type 1		Type 2		Type 3	
		Day 35	Day 49	Day 35	Day 49	Day 35	Day 49
Intramuscular	100%	158 (90–277)	354 (205–611)	322 (225–462)	256 (167–393)**	397 (223–707)	388 (227–663)
	10%	85 (38–190)	354 (167–750)	147 (69–312)	203 (96–430)	149 (59–377)	142 (70–287)
	20%	181 (112–293)	256 (132–496)	173 (95–316)	177 (96–327)	141 (81–243)	115 (52–255)
	40%	78 (38–161)	194 (130–289)	128 (68–242)	109 (62–191)**	169 (81–355)	204 (110–378)
Intradermal	10%	69 (31–152)	92 (38–223)	63 (20–199)	104 (40–274)	44 (18–105)	107 (43–267)
	20%	117 (59–231)	281 (153–514)	169 (98–290)	616 (340–1114)*.***	190 (103–348)	406 (250–662)*
	40%	308 (156–609)*	758 (435–1322)*	250 (175–358)	281 (163–485)*	119 (45–17)	446 (313–635)*

Neutralizing titers are presented as geometric mean titer (95% confidence interval).

* $P < .05$, *t* test, immunization with equal dose of vaccine, intradermal vs intramuscular route within respective serotype/day.

** $P < .05$, *t* test, intradermal vs equal or higher dose given through intramuscular immunization route.

An important difference between previously published human studies and the results described here is that both the 20% and the 40% intradermal groups had antibody titers similar to the 100% intramuscular group. All previous studies showed decreased antibody titers in 20% intradermal vs 100% intramuscular groups (reviewed in [8]). This difference could be explained by species differences, in that the maximally beneficial IPV dose might be smaller in rats than in humans. Alternatively, this could be because of our use of a different intradermal delivery device. We used the NanoPass MicronJet600, a microneedle device designed to target the dermis, whereas 5 of the 6 published human studies used a needle-free injector, and the sixth used a standard needle and syringe. The results of our current human trial, in which we also use the MicronJet600, when compared to the published studies, will help determine if the intradermal delivery device utilized impacts the immunogenicity of fractional-dose intradermal IPV.

In conclusion, our results suggest that fractional dose of IPV given intradermally can be equivalent to full IPV dose given intramuscularly in rats, both in terms of seroconversion and antibody titers, if the fractional dose is not too low. They also suggest that intradermal delivery devices may affect the outcome of immunization. These results only provide a proof of concept, given the significant differences between the immune systems of rats and humans. Further human studies are needed to determine if our findings could have practical implications for vaccination of children in the developing world, where fractional dose intradermal IPV would most likely be utilized.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Financial support. The work was funded by the intramural research program of the US Food and Drug Administration; the National Institute of Allergy and Infectious Diseases (grant number K23 AI093678); and the Doris Duke Charitable Foundation (grant number 2012061) to S. T.

Potential conflicts of interest. E. K. and Y. L. are employed by NanoPass Technologies Ltd. All other authors report no conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Global Polio Eradication Initiative. Polio eradication and endgame strategic plan 2013–2018. Geneva, Switzerland: WHO, 2012.
2. 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.
3. Martin J. Vaccine-derived poliovirus from long term excretors and the end game of polio eradication. *Biologicals* 2006; 34:117–22.
4. Jenkins HE, Aylward RB, Gasasira A, et al. Implications of a circulating vaccine-derived poliovirus in Nigeria. *N Engl J Med* 2010; 362: 2360–9.
5. Strategic Advisory Group of Experts (SAGE) on Immunization. Meeting of the Strategic Advisory Group of Experts on immunization, April 2012—conclusions and recommendations. <http://www.who.int/wer/2012/wer8721/en/>. Accessed 18 November 2014.
6. Asturias EJ, Dueger EL, Omer SB, et al. Randomized trial of inactivated and live polio vaccine schedules in Guatemalan infants. *J Infect Dis* 2007; 196:692–8.
7. Strategic Advisory Group of Experts (SAGE) on Immunization. Meeting of the Strategic Advisory Group of Experts on immunization, April 2014. http://www.who.int/immunization/sage/meetings/2014/april/report_summary_april_2014/en/. Accessed 18 November 2014.
8. Nelson KS, Janssen JM, Troy SB, Maldonado Y. Intradermal fractional dose inactivated polio vaccine: a review of the literature. *Vaccine* 2012; 30:121–5.
9. Cadorna-Carlos J, Vidor E, Bonnet MC. Randomized controlled study of fractional doses of inactivated poliovirus vaccine administered intradermally with a needle in the Philippines. *Int J Infect Dis* 2012; 16: e110–6.
10. Soonawala D, Verdijk P, Wijmenga-Monsuur AJ, et al. Intradermal fractional booster dose of inactivated poliomyelitis vaccine with a jet injector in healthy adults. *Vaccine* 2013; 31:3688–94.
11. Laurent A, Mistretta F, Bottiglioli D, et al. Echographic measurement of skin thickness in adults by high frequency ultrasound to assess the appropriate microneedle length for intradermal delivery of vaccines. *Vaccine* 2007; 25:6423–30.
12. Levin Y, Kochba E, Kenney R. Clinical evaluation of a novel microneedle device for intradermal delivery of an influenza vaccine: are all delivery methods the same? *Vaccine* 2014; 32:4249–52.
13. Hung IF, Levin Y, To KK, et al. Dose sparing intradermal trivalent influenza (2010/2011) vaccination overcomes reduced immunogenicity of the 2009 H1N1 strain. *Vaccine* 2012; 30:6427–35.
14. National Research Council. Guide for the care and use of laboratory animals. 8th ed. Washington, DC: The National Academies Press, 2011.
15. World Health Organization. Manual for the virological investigation of poliomyelitis. Geneva, Switzerland: WHO, 1997.