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Repeated fractional intradermal dosing of an inactivated polio vaccine by a single hollow microneedle leads to superior immune responses

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ABSTRACT

The purpose of this study was to investigate the effect of various repeated fractional intradermal dosing schedules of inactivated polio vaccine serotype 1 (IPV1) on IPV1-specific IgG responses in rats. By utilizing an applicator that allowed for precisely controlled intradermal microinjections by using a single hollow microneedle, rats were immunized intradermally with 5 D-antigen units (DU) of IPV1 at 150 μm skin depth. This dose was administered as a bolus, or in a repeated fractional dosing schedule: 4 doses of 1.25 DU (1/4th of total dose) were administered on four consecutive days or every other day; 8 doses of 0.625 DU (1/8th of total dose) were administered on eight consecutive days; or 4 exponentially increasing doses (0.04, 0.16, 0.8 and 4 DU), either with or without an exponentially increasing CpG oligodeoxynucleotide 1826 (CpG) dose, were administered on four consecutive days. All of these fractional dosing schedules resulted in up to ca. 10-fold higher IPV1-specific IgG responses than intradermal and intramuscular bolus dosing. IPV1 combined with adjuvant CpG in exponential dosing did not significantly increase the IPV1-specific IgG responses further, which demonstrated that maximal responses were achieved by fractional dosing. In conclusion, repeated fractional intradermal IPV1 dosing leads to superior IPV1-specific IgG responses without the use of adjuvants. These results indicate that a controlled release delivery system for intradermal IPV1 delivery can potentiate IPV1-specific IgG responses.

1. Introduction

The World Health Organization has initiated a plan to eradicate poliomyelitis completely. Extensive vaccination programs play a major role in this plan [1]. Currently, inactivated polio vaccine (IPV) and oral polio vaccine (OPV) are both used to prevent poliomyelitis. IPV is expensive, because of high production costs and the requirement of a relatively large dose for protection [2]. However, because OPV is based on attenuated live polio virus strains, it carries the risk that it may revert to wildtype poliovirus and cause outbreaks of vaccine-derived polioviruses. Sometimes this results in vaccine-associated paralytic poliomyelitis in vaccinated individuals or their contacts [3,4]. For that reason, the strategy of the World Health Organization is to replace OPV by IPV [1]. Simultaneously, several strategies to reduce costs for IPV immunization are investigated, including dose sparing strategies, such as adjuvanting IPV [2]. Studies have been conducted focusing on the IPV-specific immune response-enhancing effects of adjuvants, such as aluminum salts, calcium phosphate, oil emulsions, chitosan, vitamin D, CpG oligodeoxynucleotides, stearyl tyrosine, liposomes and others [5–13]. Although adjuvants may increase (antigen-specific) immune responses and may result in antigen dose sparing, they can also cause side-effects, may be expensive and may introduce vaccine formulation stability problems [14]. Therefore, avoidance of adjuvants in vaccine formulations can increase vaccine safety and stability and reduce costs.

As an alternative strategy for dose sparing, other delivery routes than the intramuscular route have gained much interest in recent years [2]. The intradermal delivery route has been regarded as very attractive, as it has been reported that intradermal immunization may result in increased immune responses, owing to the unique immunological properties of the densely packed skin dendritic cells [15]. Indeed, IPV dose sparing has been reported for intradermal IPV immunization of infants by intradermal injections with conventional needles [16]. However, intradermal injections with conventional needles are difficult

Abbreviations: CpG, CpG oligodeoxynucleotide 1826; DU, D-antigen units; ID, intradermal; ID-bolus, intradermal bolus immunization; IM, intramuscular; IM-bolus, intramuscular bolus immunization; IPV, inactivated polio vaccine; IPV1, inactivated polio vaccine serotype 1; OPV, oral polio vaccine; PBS, phosphate buffered saline; VN, virus neutralization.

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to perform and painful. Therefore, there is a need for alternative intradermal immunization methods. Microneedle-mediated vaccine delivery is a relatively new strategy to intradermally deliver vaccines in a minimally invasive and potentially pain-free manner [17]. For this reason, microneedle-mediated intradermal IPV immunization was examined in rats, rhesus macaques and humans [6,13,18–23]. Previously, we developed hollow microneedles and an applicator for hollow microneedles [18], that allows precisely controlled intradermal microinjections [13], taking full advantage of the unique immunological properties of the skin. Furthermore, prolonged delivery of a peptide antigen has led to increased immune responses [24]. However, it is unknown whether prolonged delivery of a vaccine antigen that is used in vaccine programs will lead to increased immune responses as well and whether precise dosing in time is affecting antigen-specific IgG responses.

In the present study, the IPV dose was divided over multiple days using various multi-day dosing schedules and injected into the skin by hollow microneedle-mediated microinjections. In this way, the IPV dose was delivered into the skin in repeated fractional doses. We present that superior IPV-specific immune responses by immunization with repeated fractional doses are induced, without the use of adjuvants and taking advantage of the potentially pain-free IPV delivery into skin by hollow microneedles. This demonstrates that a controlled release delivery system which delivers IPV1 intradermally over multiple days may substantially increase IPV1-specific IgG responses.

2. Materials

Concentrated sulfuric acid 95–98% and hydrofluoric acid 49% w/w were purchased from Sigma-Aldrich, Zwijndrecht, the Netherlands. Silicone oil AK350 was ordered at Boom, Meppel, the Netherlands and 20-μm inner diameter polyimide coated fused silica capillary at Polymicro, Phoenix, USA. Female Wistar Han IGS rats (Crl:WI(Han), strain code 273, 175–225 g) were obtained from Charles River, Saint-Germain-sur-l'Arbresle, France. Isoflurane 100% w/w was purchased from Abbott, Maidenhead, UK. Phosphate buffered saline (PBS: 163.9 mM Na^+, 140.3 mM Cl^-, 8.7 mM HPO_4^{2-} and 1.8 mM H_2PO^{4-}, pH 7.4) was ordered at B. Braun Melsungen, Melsungen, Germany and CpG oligodeoxynucleotide 1826 (CpG) at Invivogen, Toulouse, France. 2.5 mL Vacuette® Z-serum-separator clot-activator premium-tubes were obtained from Greiner Bio-One, Alphen a/d Rijn, the Netherlands. Monovalent IPV type-1 (IPV1) was kindly provided by Intravacc, Bilthoven, the Netherlands.

3. Methods

3.1. Applicator and fabrication of hollow microneedles

Hollow microneedles were produced in-house as previously described [13,18]. In short, the inner lumen of 20-μm inner diameter polyimide-coated fused-silica capillaries was filled with silicone oil overnight in vacuo at 100 °C. These capillaries were wet-etched into hollow microneedles, by immersing one side (±10 mm) of the capillaries in 49% hydrofluoric acid for 4 h. Next, the polyimide coating was removed by immersing the etched capillaries in concentrated sulfuric acid at 250 °C for 5 min, which resulted in hollow microneedles. An in-house designed hollow microneedle applicator [18], allowing for controllable injection rate, volume and depth [13], was used for intradermal microinjections by these hollow microneedles.

3.2. Immunization studies

Animal studies were performed obeying the guidelines and regulations enforced by Dutch law and the animal ethic committee. These studies were approved by the "Dierexperimentencommissie Universiteit Leiden (UDEC)" under number 12084. Female Wistar Han rats (weight on arrival 175–225 g) were housed in groups of five in a controlled environment subjected to guidelines of the animal facilities of the Leiden Academic Centre for Drug Research, Leiden University. The animals were randomly assigned to immunization groups (10 animals per group). Animals were anesthetized with isoflurane prior to shaving, blood withdrawal and immunization. Animals that were assigned to intradermal immunization groups were shaved (an area of 4 cm^2 on the left posterior flank) prior to the intradermal microinjection. For intradermal immunization, a single hollow microneedle was applied by using an applicator for hollow microneedles to perform microinjections into the skin at a pre-defined skin injection depth, rate and volume of 150 μm, 20 μL/min and 10 μL, respectively. Control groups received intramuscular injections of 100 μL per hind leg (200 μL in total).

Two immunization studies were performed to investigate effects of repeated fractional IPV1 dosing. The dosing schedule of the first study is presented in Table 1. In detail, all groups (except the PBS treated group) received a total IPV1 dose of 5 D-antigen units (5 DU, 1/8th human dose) per immunization course, injected either by intradermal microinjections or by intramuscular injections by a hypodermic 26G needle. The total IPV1 dose was either given at once (bolus), was divided into four 1/4th doses and delivered over four consecutive days (4 × 1/4th (4 d)) or was divided into four exponentially increasing doses and delivered over four consecutive days (Exp). The latter dosing schedule was also performed for IPV1 doses combined with adjuvant CpG (Exp-CpG). The total CpG dose was 10 μg and this dose was also divided into four exponentially increasing doses over four consecutive days, similar to the IPV1 dose. A bolus dose was delivered either by a single hollow microneedle-mediated intradermal microinjection (ID-bolus) or by a single conventional intramuscular injection by a hypodermic needle (IM-bolus). These dosing schedules were performed on day 0 as prime immunization course and these same dosing schedules were repeated, starting on day 21, for a booster immunization course. Blood samples of each individual animal were collected on 7-day intervals, which started on day 0 and ended on day 42. Blood samples were collected in 2.5 mL Vacuette® tubes and stored on ice before centrifugation at 2000*g* for 10 min to isolate serum, which was stored at −80 °C prior to analysis.

A second immunization study was performed to further examine the effect of repeated fractional IPV1 dosing. The dosing schedule of the

Table 1
Dosing schedule of the first immunization study.

Delivery route	Group name	IPV1 dose (DU) on day				IPV1 dose (DU)	
		0 and 21	1 and 22	2 and 23	3 and 24	Per immunization	Total
Intra-dermal	ID-Bolus	5	–	–	–	5	10
	4 × 1/4th (4 d)	1.25	1.25	1.25	1.25	5	10
	Exp	0.04	0.2	0.8	3.96	5	10
	Exp-CpG[a]	0.04	0.2	0.8	3.96	5	10
Intra-muscular	IM-Bolus	5	–	–	–	5	10
	PBS	0	–	–	–	0	0

The IPV1 dose delivered during each immunization course (prime and boost) was 5 DU. This dose was delivered as single injection (bolus) or was delivered over 4 days (4 d). The total IPV1 dose delivered in this study after two courses of immunizations (prime and boost) was 10 DU.

[a] The IPV1 dose was combined with an exponentially increasing CpG dose of 0.08, 0.32, 1.6 and 8 μg, respectively (total CpG dose 10 μg).