Novel formulations and delivery strategies for inactivated polio vaccines : new routes with benefits

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General introduction and thesis outline

POLIO

POLIOMYELITIS IS A HIGHLY INFECTIOUS DISEASE CAUSED BY THE POLIOVIRUS, A HUMAN ENTEROVIRUS BELONGING TO THE PICORNAVIRIDAE FAMILY. THE POLIOVIRUS IS A SMALL (30 NM) NON-ENVELOPED, POSITIVE STRAND RNA VIRION WITH A PROTEIN SHELL CALLED A CAPSID. THERE ARE THREE SEROTYPES OF WILD POLIOVIRUS (TYPE 1, TYPE 2 AND TYPE 3), EACH WITH A SLIGHTLY DIFFERENT CAPSID PROTEIN. THE MOST OFTEN SPREAD BY FECAL-ORAL ROU
MOUTH AND MULTIPLIES
ARE AS

INTRODUCTION

Polio

Poliomyelitis is a highly infectious disease caused by the poliovirus, a human enterovirus belonging to the Picornaviridae family. The poliovirus is a small (30 nm) non-enveloped, positive strand RNA virion with a protein shell called a capsid. There are three serotypes of wild poliovirus (type 1, type 2 and type 3), each with a slightly different capsid protein. The virus is most often spread by fecal-oral route; it enters through the mouth and multiplies in the intestine. Most polio infections are asymptomatic or lead to mild symptoms causing minor illness. In less than 1% of the cases, the poliovirus can invade the central nervous system, which may cause irreversible paralysis and even death in case breathing muscles become affected. Polio-infected individuals shed poliovirus into the environment for several weeks, where it can spread rapidly through the population, especially in lower developed areas with poor sanitation. Polio can strike at any age, but mainly affects young children. Since there is no cure for poliomyelitis, the only way to combat polio is by prevention through vaccination.

Polio vaccines

The first vaccine against polio was developed in the 1950s by Jonas Salk. This inactivated polio vaccine (IPV) consists of formalin-inactivated (killed) poliovirus strains of all three serotypes (type 1, Mahoney or Brunhilde; type 2, MEF-1; and type 3, Saukett strains). The vaccine is given via intramuscular or intradermal injection and confers protection against disease via the induction of serotype-specific antibodies in the blood. In the 1960s, Albert Sabin developed the oral poliovirus vaccine (OPV), a trivalent vaccine based on attenuated poliovirus (Sabin) strains. Major advantage of this orally administered vaccine is the induction of antibodies both in the blood and, like in natural infection, locally in the gut. Since the live-attenuated OPV is able to replicate in the intestine, it is able to elicit effective immunity at the primary site of poliovirus entry. Moreover, in areas of poor hygiene and sanitation, OPV vaccination can result in unintentional transfer of the vaccine to people who have not been vaccinated. Therefore, the use of OPV can rapidly stop person-to-person transmission and interrupt further spreading of the (wildtype) poliovirus through the whole community.

Both vaccines, OPV and IPV, are considered as safe, but in extremely rare cases the live-attenuated vaccine-virus in OPV might cause paralysis, the so-called vaccine-associated paralytic polio (VAPP). Moreover, also very rarely, when there is insufficient coverage in the community, the vaccine-virus may be able to circulate and might revert in a form with similar

transmissibility and neurovirulence as wild polioviruses. These circulating vaccine-derived polioviruses (cVDPVs) can cause new polio outbreaks. A major advantage of OPV is its affordability for low- and middle-income countries. Since IPV is not a live vaccine, it has no risk of VAPP, but IPV is more than five times more expensive than OPV. Thereby, conventional intramuscular vaccine administration using needle and syringes requires trained health workers, as well as sterile injection equipment and procedures to avoid re-use of needles or needle-stick injuries. Advantages and disadvantages of both OPV and IPV are listed in **table 1**.

Table 1 Pros and cons of both polio vaccines: the live-attenuated oral polio vaccine (OPV) versus inactivated polio vaccine based on Salk strains (Salk IPV).

	OPV	Salk IPV
Pros	Highly affordable Ease of administration Mucosal immunity (intestinal sIgA)	Safe (no risk of VAPP[1]) Relatively stable High efficacy (1-2 dose)
Cons	Risk of VAPP[1] Risk of circulating VDPV[2] Very poor thermostability	High costs No mucosal immunity Trained health personnel needed to administer

[1] *VAPP – vaccine-associated paralytic polio*
[2] *VDPV – vaccine-derived polioviruses*

Polio eradication

Since the introduction of the Global Polio Eradication Initiative (GPEI) in 1988, the incidence of polio has decreased by more than 99.9%. In the late 1980s, polio paralyzed more than 1000 children every day, whereas only 22 cases of wildtype and 91 cases of vaccine-derived polioviruses were reported last year (2017) (**Figure 1**). However, polio remains endemic in three countries that have never stopped polio transmission: Afghanistan, Nigeria and Pakistan.

Of the three wildtype polioviruses, two of them are eradicated. The last case of type 2 was reported in 1999 and the most recent case of wildtype poliovirus type 3 dates to November 2012. However, stopping the last polio cases to reach global polio eradication proved to be challenging due to conflicts, political instability, hard-to-reach populations, and poor infrastructure. Therefore, in 2013 the GPEI launched a comprehensive strategic polio endgame plan that describes several steps to reach and maintain a polio-free world. The first objective of the plan is to discontinue the transmission of all wildtype poliovirus, but also to rapidly stop new outbreaks due to circulating VDPVs. This should be reached

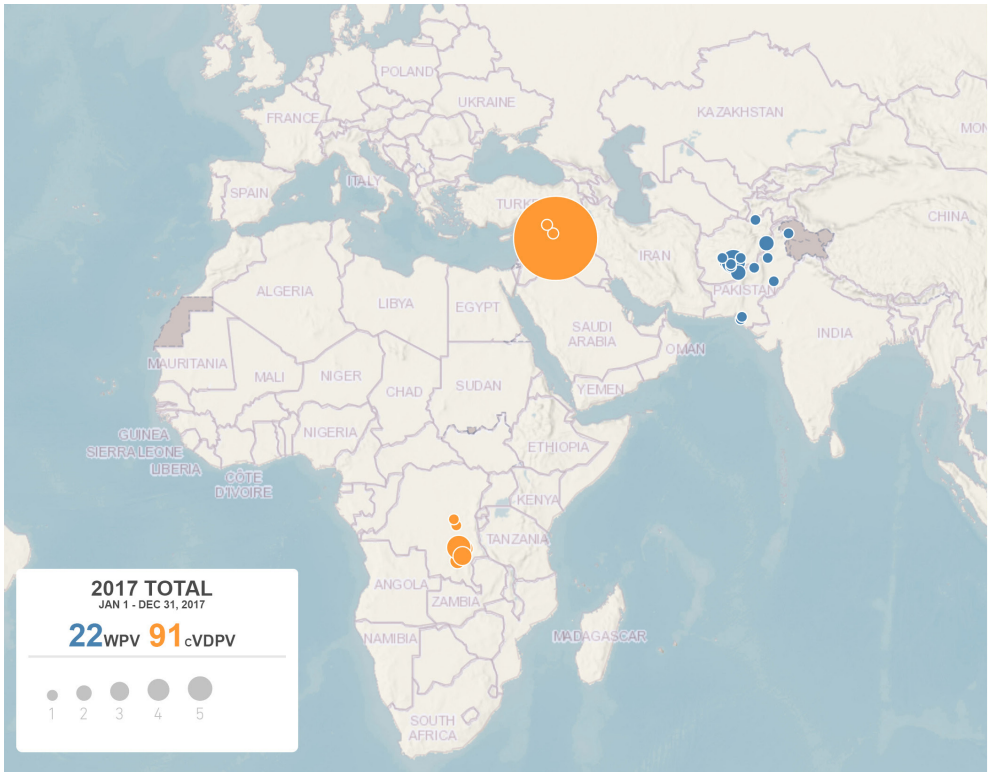

Figure 1 Geographical distribution of polio cases reported in 2017. Both wildtype polioviruses (WPV, blue dots) and circulating vaccine-derived polioviruses (cVDPV, orange dots) are shown.
source: www.polioeradication.org

by enhancing global polio surveillance, improving the quality of OPV campaigns in the remaining endemic countries and ensuring rapid outbreak response. The second objective includes strengthening of immunization programs and the phased withdrawal of OPV in order to hasten the interruption of all poliovirus transmission. As wildtype poliovirus type 2 was eradicated since 1999 and main cause of VDPV outbreaks is currently the type 2 component of OPV (91 VDPV type 2 cases reported), every country using trivalent OPV switched to bivalent OPV to reduce the risks of new circulating VDPVs. Thereby, the introduction of at least one dose of trivalent IPV into all routine immunization programs is part of the plan to strengthen immunization systems, especially in areas of highest risk. All these efforts resulted in the current situation with fewer cases reported from fewer areas in fewer countries than ever before (Figure 1).

Another aim of the Polio Eradication & Endgame Strategic Plan encompasses the certification of all regions to be polio-free and the guarantee that all poliovirus stocks are safely contained. Strict requirements for safe handling and biocontainment of polioviruses, retained

at only a small number of facilities, are essential to minimize the risk of reintroduction of the poliovirus in the post-eradication era. Currently, the GPEI is developing a post-certification strategy to ensure maintenance of a polio-free world after complete eradication. Polio legacy planning, as fourth objective of the endgame plan, and its subsequent implementation into public health programs should ensure that the world learns from all investments made in polio eradication for future health care.

While the program had many successes, eradication goals were not reached yet due to both external (e.g., growing conflict areas) and internal factors (e.g., suboptimal management, inappropriate surveillance, tight IPV supply). As the virus' prevalence is declined to very limited parts of the world, the eradication of polio depends on the success of health workers to identify and vaccinate every high-risk child, even living in dense urban environment, in extremely remote and hard-to-reach area, or being on the move.

Towards and beyond polio eradication

The inclusion of IPV into all global routine immunization programs and the possible eradication of polio spur the need for improved and affordable IPV formulations. Important variables for the development of new IPV formulations are the route of administration, the selection of adjuvants, the vaccine formulation, and the use of (non-invasive) delivery methods. Ideally, a new generation of IPV have the benefits of OPV and should therefore be easy to administer, provide mucosal immunity, and be affordable for low-income countries. IPV formulations with improved thermostability that can be kept outside the cold chain may simplify logistics and increase vaccine availability in remote areas. Furthermore, the ideal polio vaccine should be safe to manufacture and have a long shelf-life with both characteristics being even more critical in the period after polio eradication, respectively to reach biosafety goals and for stockpiling purposes.

Towards polio eradication and also in the period thereafter, there will be a market for better IPV formulations. Besides, it is important to build up stocks of a polio vaccine that can be used as outbreak intervention in case of reemergence of poliovirus in the post-eradication era and even in the period post-vaccination.

THESIS SCOPE AND OUTLINE

The aim of this thesis was to develop improved formulations and novel delivery strategies for polio vaccination using IPV as staring point. In chapter 2, the current status of alternative polio vaccine delivery strategies is provided. The feasibility of these strategies is discussed by highlighting challenges, hurdles to overcome, and formulation issues relevant for optimal vaccine delivery.

Chapter 3 describes the development of a dried IPV having minimal loss during the lyophilization process and improved stability when compared with the conventional liquid IPV. A certain thermostable vaccine formulation should allow distribution and storage at unrefrigerated conditions, at least long enough for their transport to remote areas. In chapter 4 the potency of lyophilized IPV was evaluated. Moreover, an approach to obtain a hexavalent vaccine by reconstituting lyophilized IPV with liquid pentavalent vaccine, which contains diphtheria toxoid, tetanus toxoid, whole cell pertussis, Haemophilius influenza type B and hepatitis B (DTwP-Hib-HepB), is described.

The potential of the Bioneedle-technology as syringe-free alternative delivery system for polio vaccination is described in chapter 5. Bioneedles are small biodegradable mini-implants that can be filled with antigen followed by a lyophilization process. After subcutaneous delivery, the implant dissolves and the antigen releases. Antigenicity of IPV when formulated in Bioneedles was assessed, both directly upon preparation and after elevated stability testing. Further, we evaluated the immunogenicity of IPV-filled Bioneedles in rats and the residence time at the site of administration.

Mucosal tissues are attractive administration and target sites for vaccination due to their large surface and immunological competence. In chapter 6, the characteristics of and approaches for sublingual and buccal vaccine delivery are described and compared with other mucosal vaccine delivery routes. Besides, this chapter highlights promising developments in the search for vaccine formulations, including adjuvants and suitable dosage forms, which are likely critical for the design of a successful sublingual or buccal vaccine.

Chapter 7 describes the potential of polio vaccination via mucosal surfaces using IPV based on the attenuated Sabin strains. It was investigated whether intranasal or sublingual vaccination with sIPV is able to elicit functional systemic immunity (serum) as well as local immune responses at different mucosal sites. The need of an adjuvant for polio vaccination via mucosal routes was examined as well by testing sIPV in combination with the mucosal

adjuvant cholera toxin. For the induction of protective immunity upon sIPV vaccination under the tongue, the development of novel oral dosage forms that facilitate antigen uptake by the oral mucosa may be required. In **chapter 8** the possibility to make polymer-based films containing trivalent sIPV suitable for oromucosal vaccination was evaluated. Different film forming polymers were selected from literature and tested in combination with excipients that stabilize the antigen during the drying process in order to obtain a sIPV-containing oral film formulation with minimal loss of antigenicity.

Chapter 9 summarizes the results and conclusions of this thesis. Moreover, all different aspects of this thesis are discussed and perspectives of improved formulations and alternative delivery strategies for polio vaccination are given.