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Developments in the production and quality control of poliovirus vaccines – Historical perspectives

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Abstract

Using virus grown in monkey kidney cells, Salk and his colleagues developed an inactivated poliovirus vaccine (IPV) in 1952. A large-scale field trial showed the vaccine to be safe and highly immunogenic in children, but soon after the vaccine became generally available in 1955, cases of paralytic disease were reported in recipients. Investigations showed that almost all the cases occurred in children who had received vaccine from one particular manufacturer. Extensive studies attributed the disaster to problems with inactivation. Addition of a Seitz filtration step midway during formalin inactivation and extension of the inactivation period resulted in a safe vaccine. No further paralytic cases were observed following the use of several hundred million doses of this improved vaccine. Thus, IPV was safe and caused a dramatic decline in the incidence of poliomyelitis in countries where it was used. A second generation IPV is produced in fermentors using well-characterized cell strains or continuous cell lines.

The major breakthrough in the development of live poliovirus vaccine was the application of tissue culture methods for virus attenuation. By 1959 several candidate live oral poliovirus vaccines (OPV) had been developed. These were clinically tested in millions of individuals and found to be safe and effective. Since the attenuated virus strains developed by Koprowski and Cox were more neurotropic in monkeys than the Sabin strains, only the latter was licensed in the USA in 1961 and endorsed shortly after by the World Health Organization (WHO). The widespread use of Sabin's OPV in many countries hastened the development of International Requirements by WHO for OPV in 1962 to define the criteria that ensured the uniformity of batches produced by different manufacturers. These have been updated continuously in light of new information and quality control procedures. Extensive field trials have shown the risk of OPV associated polio to be less than 0.3 per million doses administered.

Keywords: Inactivated poliovirus vaccine; Live oral poliovirus vaccine; Preparation of poliovirus vaccines; Quality control testing of poliovirus vaccines

1. Introduction

In 1949 Enders et al. [1] discovered that polioviruses could be propagated in human cells by using fibroblasts grown from the skin and muscle tissue of infants who had died soon after birth, opening a new era in modern virus vaccinology. Poliovirus was soon found to propagate in cells from a variety of tissues from both humans and nonhuman primates. The monkey kidney became the source of tissue for vaccine production [2].

2. Inactivated poliovirus vaccines (IPV)

2.1. First generation IPV

Several technical developments such as preparation of homogenous monkey kidney cell suspensions by trypsinization and their successful propagation on the inner wall of various culture flasks in monolayer form opened the door to prepare large volume of poliovirus suspensions of all three virus serotypes. The use of a synthetic culture medium (M199) resulted in a pure trivalent polio vaccine in which poliovirus was inactivated by 1:4000 formalin and caused few or no adverse reactions in vaccine recipients [2].

The trivalent polio vaccine was found by Salk and coworkers to be safe and immunogenic, first in monkeys and later in humans [2]. Several manufacturers started to produce

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the new inactivated poliovirus vaccine and by early 1954 the laboratory and clinical data were considered sufficient to conduct large-scale field trials in humans. Under the leadership of Thomas Francis at the University of Michigan School of Public Health, the largest clinical experiment of its kind up to that time was initiated with the new IPV. Trial participants included a total of 1.8 million children in communities from all parts of the United States and several from Canada and Finland [2]. The trial involved both observed controls and, in some areas, placebo controls. The results were presented on April 12, 1955 by Francis [3]. The conclusions were that the vaccine was safe and effective at a level of 60–70% for type 1, and at least 90% for types 2 and 3. The effectiveness could be correlated with potency, as measured by antibody response in children. On the basis of the evidence from the trial and the data presented by the manufacturers, the IPV of six producers (Ely Lilly, Park Davis, Wyeth, Pitman-Moore, Cutter and Connaught) was licensed shortly after the announcement of the field trial results [2].

Interest in the new vaccine was high and many communities organized specific vaccination programs. However, not long after the vaccine had become generally available, cases of paralytic disease were reported in recipients [2]. Epidemiologic investigation revealed that almost all the cases occurred in children who had received vaccine made by the same manufacturer – Cutter. On further investigation, it was established that two vaccine pools distributed in eight filling lots were implicated. A total of 260 cases of poliomyelitis were identified as being caused by the Cutter vaccine. Of these, 94 were in vaccinees, 126 in family contacts, and 40 in community contacts. Of the 260 cases 192 were paralytic and 10 deaths were observed among bulbar paralytic patients [4–6].

The US Public Health Service immediately suspended vaccination, recalled the Cutter vaccine, and launched an intensive investigation, including a careful review of the regulations governing the manufacture of vaccine and the techniques employed by the companies [7]. Extensive studies of production techniques attributed the disaster to two causes: (i) failure to remove viral clumps that could hide infectious particles, and (ii) a ''tailing-off'' of viral inactivation at low concentration of infectious virus, which meant that the virus inactivation was no longer linear [2]. Addition of a filtration step through a Seitz filter midway during formalin inactivation was the major new step taken, the result being increased *safety* but unfortunately also diminished *antigenicity* [8]. In addition, the virus inactivation period was extended and the volume of vaccine doses considerably increased in the test for residual infectivity in monkey kidney cultures *after virus inactivation*. In order to increase the sensitivity of the monkey safety test for residual poliovirus infectivity, monkeys were inoculated with 25-fold to 100-fold concentrated monovalent vaccine. Furthermore, at the time of vaccine inoculation each monkey received 200 mg of cortisone acetate intramuscularly to weaken the animal's immune system to make possible residual virus infection. Thus, the tragic events of the Cutter incidence had an enormous impact on the regulatory control not only for IPV but also for other vaccines as well. Requirements for the production and testing of cell banks, seed viruses, virus harvests and final products became more stringent, and consequently the safety and efficacy of existing and future vaccines improved considerably.

There was another event in the early 1960s that helped to improve the manufacture of virus vaccines [9]. During the early years of IPV and OPV production, some primary monkey kidney cell cultures were contaminated with simian virus 40 (SV40), a virus that causes cancer in rodents [10]. Although concerns were raised about the carcinogenic potential of the SV40 in vaccinees and their offspring, long-term follow-up studies did not support such an association [11,12]. Nevertheless, the question of whether SV40 exposure can increase cancer risk continues to be debated, and additional research is needed to accept or reject this association [9]. IPV and OPV lots produced since 1963 are SV40 free due to the stringent testing procedures introduced for the detection of simian and other adventitious viruses [13].

The first generation IPV described above was used widely in the USA, Canada, Netherlands, Sweden, France and Finland in the late 1950s and early 1960s. A total of 400 million doses were distributed in the USA and the vaccine was found to be safe and effective in all countries. However, with the licensing of Sabin's oral poliovirus vaccine in the early 1960s, the majority of countries in the Americas and Europe switched to OPV and abandoned IPV.

2.2. Second generation IPV

Several technical advances in the late 1960s and 1970s [9–13] permitted the development of an enhanced-potency IPV (eIPV) that, although based on principles of those of the first generation vaccine, differs in three important aspects:

1. The cell substrate on which the virulent seed viruses are inoculated is (i) either secondary or tertiary subcultures of kidneys from pathogen-free monkeys, (ii) human diploid cell strains, or (iii) the Vero African green monkey kidney cell line, rather than primary cultures from newly captured monkeys.
2. The cells are grown on microbeads in large fermentors in order to increase density.
3. The virus harvest is concentrated and purified *before* inactivation to increase the final antigen content.

The development of the microcarrier cell system by van Wezel in 1967 [14] and its application to large, up to 1000 L fermentors revolutionized virus vaccine production [15–18]. By using well-characterized cell strains the vaccine is free of extraneous contaminating agents. By application of ultrafiltration and various purification procedures the vaccine is concentrated and highly purified. The trivalent eIPV contains 40, 8 and 32 D units for types 1, 2 and 3, respectively. This formulation either as poliovirus vaccine alone or in combination with other vaccines such as diphtheria, tetanus and acellular pertussis, hepatitis B and/or *Hemophilus influenzae* type