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ABSTRACT

Oral poliomyelitis vaccine (OPV) is a critical part of the polio eradication programme. A high number of doses are administered each year with an impact on billions of citizens worldwide. It is therefore essential that written standards concerning OPV are up to date and widely available. The World Health Organization (WHO) publishes technical guidance on the quality, safety and efficacy of vaccines intended to assist national regulatory authorities (NRAs), national control laboratories (NCLs) and manufacturers. As part of its programme, on 20–22 July 2010 WHO convened a working group meeting to initiate the revision of the WHO recommendations on the production and control of OPV as presently outlined in the Technical Reports Series (TRS) issues Nos. 904 and 910 [1,2]. The attendees included experts from academia, NRAs/NCLs and industry involved in the study, manufacture, and authorization and testing/release of OPV from countries around the world including representatives from China, the European Union, Indonesia, Japan, Mexico, and the USA. The objective was to review the state of knowledge concerning production and control of OPV, with a focus on neurovirulence testing, to determine how the existing guidelines should be updated and what recommendations should be made for the future. The outcomes of this meeting will be taken into consideration in future revision of the WHO TRS.

WHO Working Group discussion on revision of the WHO recommendations for the production and control of poliomyelitis vaccines (oral): TRS Nos. 904 and 910. Report of Meeting held on 20–22 July 2010, Geneva, Switzerland☆

1. Introduction

The new global standard should reflect new developments in areas related to OPV including advanced scientific knowledge, the availability of novel laboratory techniques and the use of new vaccine formulations such as monovalent/bivalent OPV or inactivated polio vaccines (IPVs) based on Sabin seeds. Following current WHO policy, the new recommendations will also include guidance on nonclinical and clinical evaluation of vaccines.

The meeting was structured in a way that participants first discussed the current scientific knowledge related to the molecular biology, neurovirulence and pathogenesis of poliovirus in humans and various animal models as well as the process of development and characterization of live-attenuated vaccine strains. The establishment of tests to measure the neurovirulence of OPV preparations and how these tests are used within the regulatory framework during the batch release process were then described and discussed in detail. Lastly, both NRAs and manufacturers reviewed and compared the use of these tests in their institutions.

2. Summary of discussions

2.1. Scientific background

Despite years of study there are still numerous gaps in current knowledge of poliovirus pathogenesis. For example, it is not clear how and through what cells the virus enters the system during natural infection or how the virus invades the central nervous system (CNS) and localizes to the lower motor neurons. While theories exist to explain some of these phenomena, there are as yet no clear answers.

Sabin vaccine strains of all three serotypes are attenuated when given to humans. Relatively few mutations contribute significantly to the attenuated phenotypes of the three vaccine strains with those in domain V of the 5′ non-coding region (NCR) playing a key role. Reversion (or suppression) of some of these mutations is associated with an increased virulence in animal models and is believed to be a factor of pathogenicity in humans. Multiple independent properties of poliovirus appear to determine its neurovirulence phenotype. These properties might vary significantly from strain-to-strain as well as serotype-to-serotype. For the recipient, they include determinants of viral pathogenesis such as replication at point of entry, replication in lymph nodes, titre and duration of viraemia, ability to cross the blood-brain barrier, ability to replicate in anterior horn cells and spread within the CNS; and virus molecular properties such as reversion rates during vaccine formulation, reversion rates during infection, etc. There are also determinants for the community related to viral transmissibility and pathogenicity such as the amount and duration of viral excretion, the infectivity of excreted virus and the pathogenicity of excreted virus. It is evident that no single test can address all of these determinants so there are limits to the interpretations possible from any approach.

From a large number of studies in several laboratories, it can be concluded that the attenuating effects of particular mutations

☆ This report contains the collective views of an international group of experts, and does not necessarily represent the decisions or the stated policy of the World Health Organization. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned.

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in animal models can depend on species, genetic background and route of inoculation, and therefore make extrapolation to humans quite uncertain.

2.2. The monkey neurovirulence test (MNVT) model

During the development of live-attenuated Sabin vaccines, monkeys were used to assess virulence and to aid the selection process during the serial passages *in vivo* and *in vitro* and plaque purification procedures that led to the preparation of vaccine candidates. It was soon obvious that a test to measure the virulence of poliovirus would be critical to assure consistent production of safe vaccines. The MNVT was developed in the 1950s and required several years of optimization by fine-tuning different aspects of the test such as the monkey species to be used, the route of inoculation, the amount of virus and number of animals to be inoculated and the laboratory, clinical and statistical analyses to be performed. The introduction of a standardized procedure by WHO including the use of a reference virus for the test was critical for the establishment of a very sensitive neurovirulence test that also allowed comparison across laboratories and countries. The current test uses a single virus dilution and requires a minimum number of 11, 11 and 18 animals for serotypes 1, 2 and 3, respectively, to account for the different neurovirulence showed by the three vaccine serotypes. The size of the groups is based on the arbitrary requirement that the test should be able to detect a 2-fold difference in neurovirulence as compared to the reference virus. Inoculated monkeys are analysed both clinically and histologically and a detailed statistical analysis that includes historical data is conducted to determine whether the test vaccine is significantly more virulent than the corresponding reference virus. The MNVT has been used successfully for more than 40 years and is considered as a key contribution to the availability of a very safe vaccine that has helped to reduce the number of polio cases worldwide by more than 99%. The MNVT has benefited from international workshops and collaborative studies towards international standardization and is well executed; giving consistent results for all laboratories involved although some aspects still require further harmonization.

2.3. The transgenic mouse neurovirulence test (TgmNVT) model

More recently, a neurovirulence test for poliovirus using transgenic mice has been developed. Transgenic mice expressing the human poliovirus receptor were first developed in the late 1980s. Despite several efforts, as with models in monkeys, it has still not been possible to develop a suitable transgenic mouse model that resembles infection by the oral route in humans. The TgmNVT was first developed in the early 1990s by Dragunsky et al. [3] and was demonstrated to be a suitable model for all three OPV serotypes by a number of research and collaborative studies.

The TgmNVT was approved as an alternative to the MNVT for all three types of OPV for vaccine bulks at the Expert Committee for Biological Standardization (ECBS) meeting in October 2000 and was subsequently included in the WHO TRS 904 (for type 3) and TRS 910 (for type 1, 2 or 3) issued in 2002 and in the European Pharmacopoeia monograph for the testing of OPV monovalent bulks as a release assay since January 2006.

In 1993, based on preliminary data obtained with type 3 OPV WHO launched an international collaborative study aiming development of transgenic mouse model to replace monkey neurovirulence test for control of OPV neurovirulence. In total, the collaborative studies spanned 8 years in 5 phases, involved the performance of 206 assays on 94 different samples in 11 laboratories. The TgmNVT also required fine-tuning during the research and development phases. The test uses a particular strain of transgenic mouse (TgPVR21) and is carried out at 2 separate doses for each sample [4]. Five microlitres are injected intraspinally into each mouse which is a technique that requires great dexterity and a lot of training. The test results are based on clinical observations to determine whether the test vaccine is not significantly more virulent than the test reference. The main difference with the MNVT is there is no histological examination. As with the MNVT, the statistical analysis makes use of historical data to establish the pass/fail criteria. Most OPV manufacturers in Europe have started to move from the MNVT to the TgmNVT because of price, ethical and logistic issues. However, switching to the TgmNVT is not a straightforward process and all of the steps along the way are subject to extensive training and validation. As with the MNVT, the TgmNVT has benefited from regular workshops and collaborative studies and is well executed, giving consistent results for all laboratories. In fact, the TgmNVT is much better standardized than the MNVT in some aspects such as the animal species/strain and standard procedures used, the implementation and validation processes and the levels of control to ensure consistency and prevent a drift in clinical scoring results. These include independent observation by the responsible NRA, repeat testing annually at NIBSC of 10% of the vaccine batches produced in Europe, and the availability of independent training and proficiency testing using DVDs. It can be completed in 2 weeks for the mouse test compared to 1.5–2 months for the monkey test. The TgmNVT has been extensively used by manufacturers as part of the batch release process for OPV for serotypes 1 and 3 and to a lesser extent for serotype 2 vaccines. However, unlike the primate test, the TgmNVT does not provide a permanent record of the test in the form of histological slides which can be independently assessed or reassessed in the future, and there is less experience with the effect of mutations in the vaccine virus on the test outcome. In both the TgmNVT and MNVT the correct placement of inoculum is documented by a characteristic limb jerking during the inoculation process. However, there is no internal control for the correct placement of the inoculum in the TgmNVT whereas in the MNVT histological damage of some degree must be seen for the animal to be included as valid.

In the existing WHO guideline, the TgmNVT is accepted for release of monovalent bulks at the discretion of the NRA, but the MNVT is regarded as the gold standard, to be used in the event of disagreements or the development of new working or master seeds. One objective of the meeting was to evaluate whether the two tests could justifiably be considered interchangeable for regulatory purposes.

2.4. The mutation analysis by PCR and restriction enzyme cleavage (MAPREC) assay

The MAPREC assay is a molecular method developed by Chumakov [5] used to determine the proportion of single base mutations experimentally associated with attenuation at a given point within the viral RNA. If the calculated value of the mutation in Sabin vaccine at this site is greater than acceptable values, the vaccine will fail the MAPREC test. For each serotype, specific PCR primers are used to amplify a short segment of the genome containing the base to be quantified. Mutations in domain V of the internal ribosome entry site (IRES) are the target for this test. One of the primers contains modifications required to create a unique restriction site for enzyme digestion. The acceptable level of mutant content from a batch of vaccine determined by MAPREC is currently defined only for type 3 in the WHO TRS 904. Reference reagents used for the MAPREC test were established by WHO collaborative studies and adopted by ECBS in stages: type 3 in 1996 and 1997, type 2 in 2004 and type 1 in 2009. They consist of an International Standard (DNA), a high mutant reference virus, and a low mutant reference virus, as well as a DNA containing 100% of the mutation of interest, to control enzyme digestion.