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WHO Consultative Group: Report of a meeting on quality, safety and efficacy specifications for live attenuated rotavirus vaccines

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Summary

WHO develops international technical specifications for the quality, safety and efficacy of a wide range of biological medicines. These specifications are used by Member States as the basis of national legislation for the regulation of such products, and are used by WHO as the basis for the pre-qualification procedure for vaccines procured by UN agencies.

Rotavirus vaccines are at an advanced stage of development but there are as yet no WHO recommendations on production and quality control to provide guidance for countries or the pre-qualification process. To facilitate this process a meeting of experts was convened by WHO and PAHO/AMRO to review the scientific basis for production and quality control of rotavirus vaccines, and to discuss specific measures to assure the safety and efficacy of rotavirus vaccines.

The meeting was attended by *x* experts from *y* countries, drawn from academia, public health, national regulatory authorities and vaccine producers. Dr Roland Dobbelaer was elected to Chair the meeting and Dr Phillip Minor to act as Rapporteur. The status of rotavirus vaccine development was reported, followed by discussions of quality issues, safety issues and then efficacy issues. Requirements for national control laboratory testing were also considered as were needs for standardization of methods or reagents. The presentations given at the meeting and conclusions developed by the group are summarized in this paper.

Consensus was achieved on a proposed structure and content for WHO guidelines on the quality, safety and efficacy of rotavirus vaccines. It was agreed that existing guidance for other live virus vaccines provides a very good basis for guidance on product characterization, especially for source materials and control of production. The basis for attenuation of current vaccines or vaccine candidates is not known but, at least for the vaccines based on the Jennerian approach of using animal (bovine) rotaviruses, is likely to be multigenic.

Safety issues addressed the risk of intussusception which in humans is influenced by genetic background and age. Recent analyzes of large vaccine safety trials found that certain strains of vaccine virus were not associated with intussusception, although in these trials the first dose of vaccine was not administered to children over 3 months of age. The detailed pathogenic mechanisms are not defined but are very likely complex. Since age is a risk factor for intussusception, this may suggest that early delivery of the first dose of vaccine is desirable. Extra-intestinal wild type rotavirus has been reported as a viraemia and also in liver, cerebrospinal fluid, spleen and kidney on occasion but the significance of these observations is not clear. Virus can be shed from the nasopharynx.

In a pig model, infectivity of vaccine strains may be greater from the nasopharynx than in the faeces, in contrast to wild strains. It is not known if this is also true of humans.

Efficacy issues included the effect of maternal antibodies on vaccine take, which may mitigate against early delivery of the first vaccine dose, the importance of strain diversity in vaccine formulations, and factors which could affect vaccine efficacy or safety such as malnutrition, other enteric infections, parasitic infection or immune suppression. It was concluded that data from clinical trials conducted in one part of the world would not necessarily be predictive of vaccine efficacy in other parts and it was noted this may have implications for the WHO pre-qualification of this vaccine.

It was agreed that nonclinical evaluation of vaccines should be based on existing generic WHO guidelines. Rotavirus specific issues include the need to use oral dosing for toxicity studies and, because rotavirus was agreed to be non-neurovirulent, that there is no need for an animal neurovirulence test. The WHO guidelines on regulatory expectations for clinical evaluation were also appropriate in general; rotavirus specific issues include the need for a standard definition of severe disease as the clinical endpoint, the seasonality of natural rotavirus infections, the need for studies in diverse geographical regions, and the need to control for possible interference by factors such as other oral vaccinations, intercurrent infections and breast feeding. There is no accepted correlate of protection. Secretory IgA is the most satisfactory laboratory parameter currently available and for a period after vaccination much of the specific serum IgA is of this type, so that serum IgA levels can act as measure of seroconversion.

The need for standardization of the potency assay for release of vaccine was identified, as was a need to develop guidance on standardized approaches to post-marketing surveillance for rotavirus vaccines.

Introduction - the context

Dr Roger Glass, US CDC, presented an overview of live attenuated rotavirus vaccine development. The accelerated development and introduction of rotavirus vaccines into the global program of childhood immunizations has been a priority of WHO since 1979 and was most recently reaffirmed by the Global Alliance for Vaccines and Immunizations. This decision was based upon the epidemiology of rotavirus which infects all children worldwide during their first few years of life, and leads to diarrhea that can be mild or life threatening, and in developing countries, is responsible for more than 500,000 deaths each year. Vaccines have been identified as the prime means of prevention because improvements in water, hygiene or sanitation are unlikely to stop the spread of this disease and field studies have documented good immunity following natural infection or immunization with a variety of oral, candidate vaccines.

A reassortant rhesus-human rotavirus vaccine, Rotashield, was developed by Dr. Kapikian at the US NIH, manufactured and licensed by Wyeth Lederle, and went into national use in the United States in 1998. Nine months later, after more than

600,000 children had been immunized, the identification of a few intussusception adverse events led to the eventual withdrawal of the vaccine in the United States. The association of the vaccine with intussusception was estimated to have a risk of 1 in 11,000 children immunized and primarily affected children older than 90 days of age. Two other manufactures, Merck, and GlaxoSmithKline (GSK) have gone on to develop alternative live oral vaccines that have just completed clinical trials in more than 60,000 infants each. The GSK vaccine, Rotarix , prepared from an attenuated strain of human rotavirus has been tested in Latin America, Europe and Asia and, at the time of the meeting, had been licensed in Mexico, the Dominican Republic and Kuwait and is being considered for national immunization programs in several countries. The Merck vaccine (Rotateq) is composed of 5 reassortant bovine-human strains, designed to provide protection against the 4 major serotypes in humans. It has completed testing in the US and Europe was submitted for licensure in the US and in the EU after the meeting, in April 2005. A variety of other vaccines have been either licensed (eg. Lanzhou lamb rotavirus vaccine, China) or are under development in Brazil, India, China (using a UK bovine-human reassortant vaccine developed at the US NIH), Indonesia, (using a RV3 human neonatal vaccine strain) or in India (using 2 natural bovine-human reassortant strains from newborns and being developed by Bharat BioTechLtd.).

The most important question remaining for these live oral vaccines is whether they will work as expected in poor populations in the developing world where rotavirus remains a killer. Clinical trials of the GSK vaccine in Bangladesh and South Africa are ongoing. Other open questions include the relative safety of these new vaccines (ie. are they safer than Rotashield ?); do they protect against the full range of serotypes in circulation?; can an adequate supply be secured at an affordable price?; and can mechanisms be put in place to monitor the impact of vaccine introduction programs?

The advent of 2 new vaccines in the next year plus several other vaccines in the next 4-7 years has opened the horizon to think about the many issues that need to be resolved to bring a new vaccine to market. A wide variety of National Regulatory Authorities (NRAs) are likely to be faced with making decisions on the acceptability of candidate rotavirus vaccines in the next few years. Dr Glass therefore considered that this consultation was very timely and would open up pathways for current and future vaccines, through initiation of a process that would lead to WHO guidelines for manufacturers and staff of NRAs.

The role of WHO in developing global technical standards for quality, safety and efficacy of vaccines was described by Dr David Wood, WHO. This role fulfills, in part, the expectation of Member States for WHO to develop, establish and promote international standards for biological products. WHO products include global written standards, global measurement standards, and support to the science-base for regulations. WHO Recommendations on production and quality control of vaccines are used as the basis of national regulations in many countries and as the technical specifications against which compliance is assessed for the purposes of pre-qualification of vaccine supply by UN agencies. The standards setting process involves consultation's involving all

interested parties including regulators, manufacturers, and researchers. On the basis of such meetings, draft guidelines are developed and circulated extensively for comment – not only to the meeting participants, but also to the WHO Expert Advisory Panel for biological standardization, national regulators, and relevant industrial experts. A final draft must be considered by the WHO Expert Committee on Biological Standardization (ECBS). On the advice of the ECBS, the guidelines may become WHO recommendations. This consultation was therefore the start of a process of developing international norms and standards to assure the quality, safety and efficacy of live attenuated rotavirus vaccines. The target is to have guidelines adopted by the 2005 ECBS.

A new regulatory pathway is also being used for at least one rotavirus vaccine. Initial license applications have been made in the intended countries of use, rather than in the country of manufacture. In response, the Pan American Health Organization (PAHO) has facilitated regulatory exchanges between Latin American countries, as described by Dr Maria de los Angeles Cortes, PAHO/AMRO. She stressed that the regulatory evaluation of new vaccines represents a great challenge for NRA's of Latin American countries. NRA's are key elements in countries for ensuring the quality of vaccines. The WHO has established a procedure for assessing compliance of NRA's in six basic functions: licensing, lot release, laboratory tests, inspections, evaluation of clinical trials, and post marketing surveillance. In the PAHO Region, only two NRA's comply with the requirements of the WHO assessment. One of the main goals of the Unit of Essential Medicines, Vaccines and Health Technologies, PAHO, WHO is to support the NRA's in the Region as part of the strategic goal to decrease the incidence of immunopreventable diseases. For achieving this goal, PAHO has developed a series of activities aimed at strengthening the regulatory bodies in preparation for the introduction of new vaccines needed in the Region. The new rotavirus vaccines offered the possibility of establishing new pathways for the evaluation of vaccines. For this purpose, PAHO organized a meeting where representatives of the NRA's of eight countries involved in the licensing of the new rotavirus vaccine shared their experiences and results on the evaluation of the product file. The NRA of the country where the product is manufactured participated in this meeting. As a result of the meeting, the NRA's with weaknesses in the different areas of the evaluation benefited from the knowledge of the strongest ones. Furthermore, most of the countries recognized the need for training on evaluation of clinical trials, and post marketing surveillance, which at present is a critical factor for the evaluation of this vaccine. An additional issue is that there are legal constraints established in most of the countries, where they require previous licensure in the country where the product is manufactured. PAHO will continue organizing meetings to enable countries to discuss files submitted for assessing the new vaccines. The implication of licensing new vaccines that have not been licensed before in other countries, offers the Region the opportunity to develop knowledge and expertise on the new products. PAHO's commitment is to support the NRA's in this challenge, and the responsibility of NRA's in Latin America to benefit public health in their communities.

WHO advises UNICEF and other UN procuring agencies, regarding the acceptability, in principle**,** of vaccines from different sources for supply to these agencies.

The procedures, as described by Liliana Chocarro, WHO, involves an initial evaluation, a reassessment and continuous monitoring of performance of the vaccine in the field. The prerequisite to consider a product for prequalification is that the NRA of the producing country or of a country that licenses the product complies with six regulatory functions defined by WHO. Thereafter, the initial evaluation consists of an assessment of the Product Summary File (PSF) comprising production, control and clinical data, a site visit to verify Good Manufacturing Practice compliance and independent testing of consecutive lots. Upon successful assessment, vaccines are pre-qualified for supply to UN agencies and posted on the web at http://www.who.int/vaccines-access/quality/un_prequalified/prequalvaccinesproducers.html . The prequalification status is valid for a two or five year period, when a reassessment evaluation takes place, consisting of a review of an updated PSF, random check or specific testing of lots, monitoring for failure to meet specifications, a consultation with the NRA and a site visit to the manufacturer.

Status of rotavirus vaccine development

Dr Corine Lecomte, GSK, Belgium, described a vaccine developed by SmithKline Beecham Biologicals S.A. Rotarix is a monovalent live attenuated vaccine derived from the human rotavirus (HRV) strain 89-12. The vaccine is lyophilized and to be reconstituted with liquid diluent before administration. It is orally given, in 2 doses, from 6 to 14 weeks of age with a minimum of 1-2 months apart. Rotarix is a stable vaccine with no potency loss upon storage at +2°C/+8°C up to the end of the proposed 3 year shelf-life and no potency loss upon storage of the lyophilized product at +37°C for 7 days.

Regarding quality aspects, Rotarix is produced on a Vero cell substrate in serum-free culture conditions. The serum-free Vero cell bank was qualified according to relevant regulatory requirements in force today. Control testing of HRV seeds included extensive testing for adventitious agents and specific viruses according to HRV vaccine strain history. A monkey neurovirulence test was also performed in compliance with the European Pharmacopoeia. Vaccine seeds were genetically characterized by complete nucleotide sequence determination. The HRV vaccine testing program was based on requirements relevant for other live attenuated virus vaccines. Residuals including cellular DNA have been assessed for process validation but are not proposed to be part of routine release testing. General safety test was also carried out for consistency and is not proposed to be part of routine release for commercial vaccine lots.

The clinical profile results from world-wide safety and efficacy studies. With respect to the reactogenicity and safety profile, the vaccine is well tolerated, with no difference observed in terms of adverse event incidence as compared to the placebo. Hence two doses of Rotarix did not increase intussusception (IS) rates over placebo (no difference in IS risk, no temporal clusters). With respect to vaccine efficacy, Phase II data have shown that two doses of Rotarix were effective in protecting against hospitalization and severe

rotavirus gastroenteritis with 90% efficacy in 1st year and 85% efficacy in 2nd year. Efficacy data from large Phase III trial will be available in a near future.

GSK Biologicals' objective in terms of regulatory approach was to bring Rotarix vaccine in the regions with highest disease incidence first. Accordingly, Rotarix registration file was firstly submitted in Mexico (approved on 12-Jul-04). Submission in Europe was done as a second step (Dec-04).

Dr Alan Shaw, Merck and Co. USA, described Merck's vaccine against rotavirus gastroenteritis, RotaTeq, which was developed in collaboration with Drs. H. Fred Clark and Paul Offit of the Children's Hospital of Philadelphia and the Wistar Institute of the University of Pennsylvania. RotaTeq is a pentavalent bovine-human reassortant vaccine comprising five bovine (WC3) viruses each displaying on its surface one of the VP7 or VP4 proteins of the human rotaviruses most frequently seen in infants (serotypes G1, G2, G3, G4 and VP4 type P1). The vaccine strains are propagated in Vero cells, and the minimum dose at the end of the storage period (+4°C in liquid, ready-to-use form) is 5 X 10e6 infectious units per virus. The vaccine has been tested in over 70,000 infants in at least eight clinical studies in 11 countries. Protection against ~70% of rotavirus gastroenteritis of any severity and ~ 100% of severe disease has been consistently observed. The vaccine was not associated with intussusception in a large, double blind, placebo-controlled trial.

Since this is a live, attenuated viral vaccine, RotaTeq has been developed and tested in accordance with the compendial standards for vaccines of this type. As a consequence, the testing carried out on the seeds, cell banks, bulk vaccine and final filled container are similar to other current live viral vaccines. However, the testing and release of RotaTeq has two features that differ from other live virus vaccines. First, the vaccine has been exempted by some regulatory authorities from the requirement for monkey neurovirulence testing based on the lack of neurological involvement, the limitation of the infection to the gut, and prior experience from other laboratories showing the lack of utility of this assay. Second, since the final filled container has a mixture of five bovine viruses each carrying one gene for a human virus surface antigen, potency testing of each individual component in the mixture is not feasible by the usual plaque assay methods. Therefore, Merck have developed and validated a cell-culture based multiplex RT-QPCR system that allows the infectivity of each component to be measured based on the amplification in cell culture of the unique human gene of each reassortant.

Quality issues

Rotavirus biology relevant to vaccine quality was reviewed by Dr Harry Greenberg, University of Stanford, USA. Key attributes of the rotavirus replicative cycle include a high level of fecal shedding making antigen detection easy, a high degree of tissue tropism and host range restriction. Methods for potency assay include focus assay, plaque assay, or CCID 50 assay, and identity testing may be performed by electropherotyping, or P and G typing by PCR, or total genomic sequencing. The utility

of producing hyperimmune cross reactive antisera to double layered particles for antigen detection was highlighted. Finally, it was noted that there is a lack of in vitro and animal models to assess the virulence or attenuation of vaccine strains and that assays to predict the likelihood of intussusception were not available. The genetic basis of virulence for wild type rotavirus is mutigenic but not clearly defined and the genetic basis of attenuation of any of the current rotavirus vaccines or vaccine candidates is not known.

The control of vaccine source materials was reviewed by Dr CD Atreya, US FDA. The purpose of any viral vaccine development and its administration to humans is to safeguard the public health by preventing wild type viral infection-associated diseases. Since prophylactic vaccines are administered to a healthy, often very young, general public the standards that are applied during manufacturing and quality control have to ensure vaccine safety. Therefore, starting materials and all processes related to manufacturing must be controlled to make sure that the final vaccine product is sterile and free from adventitious agents and oncogenic agents.

The rotavirus vaccines under consideration in this meeting are all live viral vaccines. The processing of live rotaviral vaccines, like many other live viral vaccines, involves cell disruption and, if any, incomplete purification of the virus. In-process adventitious agent inactivation steps are not included for live viral vaccines, since these steps may compromise the live nature of the vaccine itself. As a result, validation of clearance of any adventitious agents may not be possible. For these reasons a comprehensive adventitious agent testing and qualification of the vaccine source materials is essential as part of the vaccine safety control. As with any viral vaccines, production of rotavirus vaccines also involve cells, virus seed and biological reagents (such as growth supplements, serum, trypsin and any virus stabilizers used in the final product). Hence, each of these vaccine source components must be tested to ensure that they are free from adventitious agents. This redundancy in testing ensures high quality control of the final product vaccine. Dr Atreya considered that control of rotavirus vaccine source materials do not require any unique or special testing, but should follow and implement all existing regulatory guidelines that are applicable to other live virus vaccines.

The control of vaccine production was reviewed by Dr Pombo, Venezuela NRA. As noted above, rotavirus vaccines are live attenuated products and therefore should be produced using a seed lot system. Specific controls are needed at all stages of the manufacturing process including the cell cultures, single virus harvest, purified bulk, final bulk, filling and containers, final product, labeling and storage.

The current WHO Technical Report Series for live attenuated vaccines are considered as a good general guideline for the establishment of quality control specifications of rotavirus vaccines but a new, specific, document will be needed for this vaccine. Such a document should include, but not be restricted to:

- a summary protocol of production and control for batch release of the vaccine

- a potency assay, preferably validated in an inter-laboratory collaborative study using international reference materials
- specifications for the genetic stability of the rotavirus vaccines
- definition of virus characteristics to enable an assessment of manufacturing consistency
- quality specifications for the diluents used to reconstitute lyophilized rotavirus vaccines

Standardization of potency assays was described by Dr Phillip Minor, NIBSC, UK. He noted that assays of live viral vaccine can use animals, as for yellow fever, but in the majority of cases they depend on the infection of cells in culture. Infection can be detected by cytopathic effect, or focus formation by immune staining, or the production of antigen, or viral nucleic acid. Cytopathic effect may be used to develop plaques, or to identify infected cultures in cell culture infectious dose assays. Cell-based assays are affected by the cell sensitivity, which is influenced by the type of cell itself, the particular cell bank and the passage number within the laboratory. The comparability of results in different laboratories is also affected by the virus being assayed, as examples, different mumps vaccine viruses or rotavirus vaccines have been found to be detected with different sensitivities in different laboratories. Assay performance can be monitored by the inclusion of a reference material in each assay. Trends in the titre of the reference indicate changes in the assay sensitivity and indicate that some modification might be needed, such as using cells of lower passage. The titre of a common reference material can also serve to compare performance in different laboratories. In some circumstances (such as yellow fever vaccine) it has been possible to improve agreement between laboratories by expressing the titres as a ratio of the titre of a reference tested at the same time. In others, such as live poliomyelitis vaccine this strategy does not improve agreement because other unidentified and non-systematic factors have a greater effect. Such factors may be difficult or impossible to identify. Collaborative studies and proficiency studies are the best way to improve agreement between laboratories and improve performance.

Quantitative PCR has recently been explored as a way of measuring virus production in assays for mumps, measles and rubella, and also for rotavirus vaccines. This involves infecting separate cell cultures with dilutions of the virus to be assayed, incubating for a period and detecting the virus RNA produced by a quantitative PCR. It has the advantage that for a multivalent vaccine there is no need to neutralize individual components, and therefore there is no need to develop neutralizing and highly specific antibody preparations, which may be difficult to do for rotaviruses. The titre is determined by comparison with a reference material tested at the same time.

Dr Minor stressed it is important in assays of live viral content that there is one specification for a particular product. He cautioned against the approach taken with yellow fever vaccine where potency is expressed in regulatory documents in mouse LD50 units, but essentially all manufacturers and regulatory laboratories set a specification based on cell culture infectivity, using a conversion factor from one to the other that they

have determined in their own laboratory. As the conversion factor varies with the cell and the mouse strain it is impossible to compare results with confidence. He concluded that the method of measuring the infectivity influences the type of specification set.

Safety issues

The comparative pathogenesis of rotavirus infections in humans and animal models (mice, gnotobiotic pigs) was reviewed by Dr Linda Saif, Ohio State University, USA to clarify the major tissue targets (small intestinal epithelial cells) for vaccine-induced protection. Information on intussusception (IS) was summarized with an emphasis on a continued need for post-licensure surveillance for all existing or new rotavirus vaccines and a need to survey background rates of IS in different regions and populations. Also targeting first doses of vaccine to younger infants to avoid the peak age for intussusception at 4-9 months was discussed.

Dr Saif proposed a new model for IS linking together the induction of acute IS by lipopolysaccharide (LPS) in mice, the use of B-lactam antibiotics as a major risk factor for IS (potentially killing gram (-) bacteria with release of LPS) and new observations that rotavirus infections can transiently induce proinflammatory cytokine (TNF alpha) and PGE_2 responses (children and pigs) as well as lymphoid hyperplasia (children and pigs). She hypothesized all of these variables potentially acting synergistically with LPS to alter intestinal motility. The occurrence of rare but naturally occurring cases of IS in pigs suggests that the pig might be a useful model to study IS, but, as noted above, at this time there are no useful models to study rotavirus IS.

Natural rotavirus infections were recently reported to cause viremia or antigenemia in children and animal models, including gnotobiotic pigs infected with a virulent (VirHRV) but not attenuated human rotavirus (Att HRV) strain. The viremia induced could explain the rare sequelae sometimes observed for rotavirus infections in children including virus RNA detection in cerebral spinal fluid in children with CNS disease, and virus antigen detection in spleen or kidney of fatal cases. Dr Saif considered that screening for viremia and extraintestinal rotavirus infections should be done for both HRV-infected and vaccinated infants.

In the gnotobiotic pig model, nasal shedding of both VirHRV and AttHRV was observed. However the AttHRV had a greater capacity for nasal than rectal virus shedding leading to speculation that some attenuated rotavirus strains could be temperature sensitive mutants with reduced virulence because of altered tissue tropisms. Of note, currently there is no evidence indicating that the major rotavirus vaccines or vaccine candidates are attenuated on the basis of temperature sensitivity although this phenotype is easy to detect. Because respiratory shedding of rotavirus has been occasionally detected in relatively (compared to fecal shedding) low amounts in children, it may be of interest to follow-up this observation for both naturally rotavirus infected and vaccinated infants and in animal models of rotavirus disease to understand its

significance for rotavirus pathogenesis, transmission (for example by the aerosol route) and immunity.

The biologic [restricted ability to replicate in the intestine or altered tissue (nasal?) tropism] and genetic basis for rotavirus attenuation was reviewed. The genetic basis for rotavirus virulence and host range restriction is likely multigenetic in nature. In various studies the contributing genes (proteins) include: gene 4 (NSP4), gene 7 (NSP3) and gene 5 (NSP1) and the genes encoding the rotavirus structural proteins VP3, VP4 and VP7. In a study using pig x human reassortant rotaviruses in pigs, virulence was linked to 4 genes; VP3, VP4, VP7 and NSP4. Consequently there is no consistent single gene marker for rotavirus virulence which appears to be multigenetic in nature. Attenuating mutations for the current live vaccines are unknown, but if they are multigenetic, then reversion to virulence is less likely.

Finally a number of concerns for the safety and efficacy of live rotavirus vaccines in developing countries were described. Efficacy to date has not been good in low hygiene settings . The concerns included:
--Protection is needed early since first illness with natural rotavirus occurs at a young age
--Higher titers of maternal antibodies are noted - if interference with live vaccine takes occurs then the window to achieve effective vaccination is narrow
--More diverse/unusual rotavirus serotypes
--Malnutrition, vitamin, mineral deficiencies are common
--Enteric infections (multiple infections) are common
--Malaria, parasites, HIV, hepatitis disease burden may be high
--Children with immunodeficiences

Strategies for monitoring and overcoming some of these were proposed. Additional research needs for rotavirus vaccines were highlighted including: assessing heterotypic immunity and emergence of new rotavirus strains post-vaccine introduction; studying the biologic and genetic basis for rotavirus vaccine attenuation; and evaluating rotavirus extraintestinal spread, intussusception and the unique variables in developing countries affecting rotavirus vaccine safety and efficacy by conducting epidemiologic studies of vaccinated children and also research on these variables using animal models of rotavirus disease. Because of the unforeseen intussusception problem with the live oral Rotashield vaccine, development of second generation non-replicating rotavirus vaccines such as rotavirus-like particles (VLPs) or DNA plasmid vaccines for induction of active or passive immunity should be encouraged and continued as alternatives to live vaccines. Dr Saif concluded this is particularly important if the current live vaccines under test subsequently pose an unacceptably high increased risk of intussusception or other safety issues.

Generic WHO guidelines for nonclinical evaluation of vaccines were reviewed by Dr David Wood, WHO. These are a new tool developed to assist regulators and the vaccine industry in planning and performing non-clinical testing, as well as in analysing and interpreting non-clinical data, and are considered to represent the current state-of-the-

art. Regarding design of non-clinical studies, Dr Wood highlighted that the route of administration should correspond to that intended for use in clinical trials. For live attenuated vaccines, special attention should be given to the stability of attenuation, whether there was a need for a neurovirulence test, and whether there was a need to undertake an environmental risk assessment study. In the context of development of guidelines for live attenuated rotavirus vaccines, the extent to which the generic guidelines apply and whether it is necessary to highlight rotavirus-specific issues to supplement the generic guidance would need to be considered.

Lessons learnt by three vaccine manufacturers, GSK, Merck and Wyeth-Lederle, in the nonclinical evaluation of their live attenuated oral rotavirus vaccines were described by Dr Georges Thiry, IAVI, USA. These vaccines are monovalent human strain, pentavalent bovine/human reassortants, and tetravalent rhesus/human reassortants for GSK, Merck and Wyeth-Lederle, respectively. In general, guidance and recommendations which apply to any live attenuated virus vaccines had been applied to these three rotavirus vaccines. These include the animal tests that are part of the preparation and release of master cell banks, master and working virus seeds, and final products. These manufacturers highlighted that extensive testing for adventitious agents had been performed. It was noted that since no limit is given by WHO or Regulatory Authorities to the concentration of high molecular weight DNA in orally administered vaccines, manufacturers have introduced steps in the production process to bring the level of high molecular weight DNA in the final product below the limit imposed for injectable vaccine (10 ng / dose). Pre-clinical toxicity was evaluated by manufacturers in mice or in rats with a full human dose of vaccine, administered orally with antacid. The meeting concurred that such toxicity evaluation was appropriate and should continue to apply.

Two manufacturers had performed a monkey neurovirulence assay to satisfy current regulations in force in some parts of the world for live attenuated vaccines. As rotavirus is not neurotropic, and neural tissue passage was not used in the derivation of any of the vaccine strains, the meeting agreed that a neurovirulence test is not relevant and recommended that it should not be further requested.

Manufacturers have fully sequenced the rotavirus vaccine genome at initial passage levels and at final product stage for characterization purposes. Rotaviruses shed by vaccinated volunteers have also been sequenced. Modifications in sequence have been reported. However, in absence of genetic markers of virulence (as noted above), the effect of modifications of the genomic sequences on attenuation are difficult to predict. Attenuation was evaluated in humans in practice, as no animal model exists.

Furthermore, since no defined animal model exists to evaluate the risk of intussusception (IS) caused by administration of oral rotavirus vaccine, two manufacturers, GSK and Merck, evaluated the incidence of IS in large scale (>60,000 subject) human Phase III studies. Whether this strategy needs to be applied to future vaccine candidates reaching phase III studies is unclear.

Finally, studies of environmental impact evaluated in animals by Wyeth and Merck were non-conclusive. Evaluation of shedding after human vaccination was included the clinical development plans.

Dr Timo Vesikari, University of Tampere, Finland reviewed data on safety issues related to rotavirus vaccines obtained through clinical evaluations. He noted that, before 1999, and until the rare association of rhesus-human reassortant vaccine (RotaShield) with intussusception was discovered, there were few clinical safety issues related to live oral rotavirus vaccines. Candidate bovine (RIT4237 and WC3) and rhesus rotavirus vaccines had been evaluated in humans for standard outcomes of safety, including diarrhea, vomiting, and fever. In short, the bovine rotavirus vaccine strains were shown to be devoid of these side effects and totally innocuous in infants ranging from neonates to 9 months of age. In contrast, rhesus rotavirus caused often febrile reactions but seldom diarrhea or other gastrointestinal symptoms.

Rhesus rotavirus vaccine, and rhesus-human reassortants which are biologically similar to rhesus rotavirus in this respect, typically cause febrile reactions 3 to 5 days after oral administration. The rate of febrile reactions depends on susceptibility of the infant population as determined by age and, more importantly, by the level of maternally acquired rotavirus antibody. The highest fever rates (79%) were observed when Swedish infants aged 4 to 12 months were given a dose of 10^6 rhesus rotavirus (a dose that was not studied further).

In Finnish infants aged 5-6 months, a dose of 10^5 of rhesus rotavirus caused fever in up to 60% of the recipients. Rhesus-human reassortant tetravalent vaccine (RotaShield) at a dose $4x10^5$ caused febrile reactions in 33% of Finnish infants aged 2-3 months, but not in neonates, who have higher levels of maternal antibody. Second and third doses of rhesus rotavirus vaccine usually do not cause febrile reactions.

Human rotavirus candidate vaccine strain 89-12 was largely but not totally attenuated for clinical reactions such as fever and diarrhea. In US infants aged 2-4 months, fever up to 19% (placebo 5%) and diarrhea 17% (placebo 9%) were observed. The present vaccine strain RIX4414 (Rotarix) was developed by plaque purification and further culturing of 10 passages. This resulted in further clinical attenuation, as determined by absence of febrile reactions and diarrhea in infants less than 3 months of age at the time of first dose of vaccine. The vaccine has not been evaluated for safety in older infants who lack maternally acquired rotavirus antibody.

It is not known if the immediate reactogenicity of RotaShield, with fever peaking at 3 to 5 days post vaccination, has any relation to the rare occurrence of intussusception, peaking at 3 to 14 days post vaccination.

The temporal association of intussusception with RotaShield vaccine was discovered in 1999 and led to the withdrawal of the vaccine in October the same year. The actual attributable risk was initially estimated at 1:4300, but this has been found to be

too high. A later consensus put the risk at 1:10.000, but even this estimate is probably too high.

Studies of Simonsen and coworkers later revealed two important aspects of RotaShield associated intussusception. Firstly, the cases occurred slightly earlier than naturally occurring intussusception (peak at 4 to 9 months of age), but were followed by a "compensatory" decrease, suggesting that RotaShield vaccination may have selected infants who were prone to development of intussusception due to other causes. Secondly, intussusceptions after RotaShield vaccination were clearly age related. Only 20% of the intussusceptions occurred in the 62% of US infants who received the first dose of RotaShield vaccine below the age of 3 months (indicated age was 2 months). In contrast, 80% of the intussusception cases were among the 38% of the infants who received the first dose of RotaShield after the age of 3 months (against the original label).

Two major new candidate rotavirus vaccines entered clinical trials at about the same time as RotaShield was withdrawn. These were the WC-3 bovine rotavirus-based reassortant vaccine of Merck&Co.,Inc. (RotaTeq®) and human rotavirus vaccine RIX4414 (Rotarix®, GSK), derived from the 89-12 strain. After initial studies of safety (for immediate vaccine reactions) and efficacy, both were evaluated for safety with regard to intussusception. The comparison was the vaccine-attributable risk of RotaShield, then set at 1:4.300, but later acknowledged to be too high.

Merck's vaccine (RotaTeq®) was studied in a trial of 70.000 infants in eleven countries, but mainly in the USA (33.000) and Finland (23.500 infants). The first dose of vaccine was administered at the age of 2 to 3 months, and two subsequent doses at 1 to 2 months intervals after the previous one. Within a 42-day observation period after any dose, there were a total of 11 cases of intussusception with a vaccine to placebo split 6 to 5, for a relatively risk of 1.6 (95% C.I. 0.4, 6:4). None of the 6 cases in the vaccine group were after the first dose of vaccine.

GSK's human rotavirus (G1) vaccine was studied in over 60.000 infants in Latin America. The vaccine was given in two doses at approximate ages of 2 months and 4 months. Within an observation period of 31 days after either dose, there were a total of 13 cases of intussusception, with a vaccine placebo split of 6 to 7. Of these, only 1 case in the vaccine group and 2 cases in the placebo group were after the first dose. Altogether, in an observation period of approximately 100 days after the first dose, 9 cases of intussusception were observed in the placebo group versus 16 cases in the placebo group, for a relative risk of 0.56.

It may be concluded that both new candidate rotavirus vaccines, bovine rotavirus based RotaTeq® and human rotavirus-derived Rotarix®, appear safe for intussusception in the age group of 3 months and under. Both also appear to carry a lower risk than the original estimate for attributable risk of RotaShield. However, as the latter was shown to be too high and revised later, it is not really possible to conclude that the new rotavirus vaccines are actually safer for intussusception than RotaShield (although they may well be).

There are anecdotal reports of intussusception occurring within 2 weeks after RotaTeq® (1 case in a 7.6 month-old infants) and after Rotarix® (2 cases in 3- and 4-month old infants), respectively. These do not allow any conclusions about a possible causal relationship, but suggest that such cases may happen if first (or subsequent) doses of these vaccines are administered to older infants, particularly at the peak age of naturally occurring intussusception (4 to 9 months of age). These findings emphasize the importance of giving all rotavirus vaccines, or at least the first doses of these, below the age of 3 months.

Efficacy issues

Rotavirus biology relevant to evaluation of vaccine efficacy was reviewed by Dr Manuel Franco, University of Javeriana, Colombia . He showed that B cells are necessary for long term robust protection against rotavirus infection. In addition the intestinal localization of these B cells is important for antiviral immunity. Thus intestinal IgA is probably the most efficient antiviral protective mechanism. In children, serum IgA can be a good surrogate for this intestinal IgA shortly after infection because some of the intestinal polymeric IgA spills over into the blood. Nonetheless, intestinal IgA seems to decay more rapidly than serum antibodies. For this reason, and the fact that 4 months after rotavirus infection rotavirus specific serum IgA is no longer polymeric, serum IgA measured after this time point, may not reflect intestinal IgA. In addition during secondary immune responses of a subset of children, a local intestinal immune response seems to develop without an increase in serum IgA. A particular case in which the presence of serum IgA does not correlate with intestinal IgA (and thus protection) is that of infection with heterologous simian rhesus rotavirus. This rotavirus has a tendency in mice to induce (when administered at low doses) a serum antibody response without inducing local antibodies or protection. In gnotobiotic neonatal piglets (probably the best animal model to study protection against rotavirus induced disease), protection against rotavirus induced diarrhea is present only if the immunizing and challenge rotavirus share at least one of the outer capsid proteins. In humans, rotaviruses with only one P serotype (P1) seem to circulate. Thus after natural infection or vaccination of children with rotavirus that bear this P serotype, a certain degree of cross-reactive protective antibodies will be induced. The capacity of human serum IgA (that is mostly directed against VP6 and not protective in piglets) to predict protection against rotavirus is probably related to the fact that this IgA is a surrogate for the presence of anti-VP4/and or anti-VP7 cross-reactive protective antibodies. In conclusion, total serum IgA measured shortly after vaccination is probably the best (but imperfect) correlate of protection against rotavirus. Total serum IgA can probably be used as a correlate of protection for most of the vaccines in development and in use. Nonetheless, the lack of a clear understanding of protective immunity to rotavirus infection, is an important limitation for the development and improvement of rotavirus vaccines and research in this area is clearly needed.

Dr Irene Perez-Schael, Instituto de Biomedicina, Venezuela, noted that several factors may affect the efficacy of oral rotavirus vaccines. These included: age of vaccine

administration, severity of rotavirus illness used as an endpoint for assessment, seasonality, developed/developing country settings, concomitant administration of RV vaccine with oral poliovirus vaccine (OPV), number of doses administered, monovalent versus polyvalent vaccine formulations and the wild-type rotavirus serotypes circulating in a population. The age at which the oral rotavirus vaccine should be given is an important issue and several factors should be taken into consideration: the vaccine should be administered to infants in the first 6 months of life but within a range of age to avoid interference from maternal antibodies with vaccine take; the vaccine should be given early enough to prevent illness which is very frequently in the first year of life in developing countries; and the vaccine should be given sufficiently early in order to prevent a possible association of the vaccine with intussusception (< 3 months of age). Potential interference from maternal antibodies or from OPV may be overcome by the administration of two or three doses. Results to date show that rotavirus vaccine is more efficacious against severe illness. In consequence, standard definitions of (a) a diarrhea episode and (b) the severity of illness (recommended as the primary endpoint of efficacy studies) are critical to facilitate objective comparison of efficacy trials. Hospitalization may not be appropriate as an endpoint because this is an a context-sensitive situation which may not reflect, in all cases, the severity of illness.

Seasonality may affect vaccine efficacy. In countries, where the virus circulates year round (tropical settings), vaccine efficacy is independent of the time of vaccine administration. Meanwhile, in those settings with a marked seasonal pattern, rotavirus vaccine efficacy was higher when it was administrated just prior to the highest period for rotavirus circulation, probably due to a booster effect. Vaccine efficacy has been observed to be lower in poor developing country settings in comparison with developed countries. It is necessary to study the performance of rotavirus vaccines in very poor countries of Africa or Asia, where the risk of rotavirus mortality is the highest. Dr Perez-Schael noted that studies in these regions are underway.

Two or three doses are necessary to broaden the spectrum of heterotypic protection and to overcome the interference with maternal antibodies and with concomitantly administration of OPV. Monovalent rotavirus vaccines (RRV, RIT 4237, Rotarix) have shown similar efficacies when compared with polyvalent RV vaccines (RRV-TV, RotaTeq). However Dr Perez-Schael highlighted that the efficacy of vaccines against rotavirus serotypes not included in vaccine formulations is unclear at the moment.

The standardization of rotavirus immune response assays was reviewed by Dr Richard Ward, Childrens Hospital Medical Center, Cincinnati, USA. Immune responses, specifically rotavirus antibody responses, have been routinely measured during all rotavirus vaccine trials. This has been done to both use these responses as markers for vaccine “take” and to identify surrogate markers of protection or “correlates of immunity”. The former has been generally a success, the latter much less so. It should also be noted that vaccine take can be assessed by analysis of vaccine excretion rates. Live rotavirus vaccines have been developed based on evidence that natural rotavirus infections elicit protective immune responses, particularly against severe rotavirus

disease. It follows, therefore, that possible correlations between rotavirus antibody responses and protection found after natural rotavirus infection will be similar to those observed after live virus immunization.

There are numerous reports that indicate natural rotavirus infections result in serum and intestinal antibody responses detectable for months if not years after a single rotavirus infection. It is also well established that rotavirus antibody levels in infected subjects, whether they be measured in the serum or stool contents, are boosted after a subsequent infection. These antibody responses, therefore, appear to be reliable measures of "take" associated with natural rotavirus infections.

How well do rotavirus antibody levels generated by a rotavirus infection correlate with protection against subsequent rotavirus illness? Studies in subjects ranging in age from infants to adults have repeatedly demonstrated general correlations between the presence of serum and intestinal rotavirus antibodies and protection against subsequent rotavirus disease after natural rotavirus infection or rotavirus challenge. The correlations between rotavirus antibodies and protection in these studies, however, are never perfect, and subjects with relatively high titers sometimes become re-infected and even severely ill upon subsequent rotavirus exposure. Therefore, it is not known whether rotavirus antibodies are one of or possibly the sole effectors of protection after rotavirus infection or are only surrogate markers of protection. If rotavirus antibodies are effectors of protection elicited after infection, what is the nature of these antibodies, e.g., are they neutralizing antibodies (NA) that are directed at serotype-specific epitopes on the VP7 and VP4 proteins? It is generally agreed that if NA against the infecting strain are present in the intestine, they will provide at least some protection. It is not known, however, whether this is the only mechanism of protection induced after rotavirus infection or if non-NA and other effectors, such as T cells, also play major or perhaps the most important roles. Some studies have supported the hypothesis that rotavirus protection is serotype-specific, others indicate that it is not.

Serum and stool rotavirus antibody responses have been effectively used as measures of vaccine "take" with all candidate live virus vaccines. Wyeth's tetravalent vaccine (TV- Rotashield) induced serum IgA and NA responses to rhesus rotavirus in >90% of vaccinees after 3 doses in multiple trials. Merck's pentavalent WC3-based vaccine (RotaTeq) elicited NA responses to its G1 component in >80% of vaccinees during a trial in Finland. GSK's monovalent human RV vaccine (Rotarix) was found to induce serum rotavirus IgA responses that varied between settings. One determinant of vaccine "take" after Rotarix vaccination has been the quantity of maternal NA in the child's blood at the time of immunization. A consistent inverse relationship between transplacental neutralizing antibodies to Rotarix and vaccine "take" following immunization has been found in countries representing all socioeconomic levels.

Serum RV IgA responses in Rotarix vaccinees have directly reflected the efficacies of this vaccine. Vaccinees that did not develop rotavirus IgA (non-responders) were about 10 times more likely to experience a subsequent rotavirus illness during the

first season than responders. Dr Ward pointed out, however, that in Latin America, protection against severe disease was approximately 86% while serum rotavirus IgA responses were observed in about 65% of vaccinees. Correlations between rotavirus antibody titers in vaccinees and protection have been less evident with the other two major vaccine candidates. With Rotashield, <20% of the subjects developed NA to any representative of the 4 human G serotypes contained in the vaccine even though protection was routinely >50% against any rotavirus illness and >70% against severe disease. Serotype-specific correlates of protection were identified but serotype-specific immunity was no more significant than heterotypic immunity, and no specific titer of any antibody analysed was a reliable indicator of protection. Although NA titers to some of the G proteins of Merck's RotaTeq vaccine have been much greater than those generated after immunization with Rotashield, no serotype-specific correlate of protection has been found. Stool IgA responses were also examined after RotaTeq immunization and stool rotavirus IgA responses correlated well with serum rotavirus IgA responses but, like serum antibody responses, they have not been correlated with protection.

Taken together, these results suggest that factors other than serum or intestinal antibody could play roles in protection. Unless the antibodies being measured are those responsible for most if not all protection, it is unlikely that the overall roles of antibodies after live virus immunization will be truly determinable by these assays. It is likely that the rotavirus antibodies being measured in these vaccine trials are one of several effectors of protection and possibly antibodies against several rotavirus proteins which are not routinely measured, or sites not routinely sampled (e.g. the intestine), will have major roles in protections.

Conclusions

An outline for WHO guidelines for quality, safety and efficacy of rotavirus vaccines was proposed as a result of the deliberations during the meeting. This would include the following elements and issues:

1. Introduction
    - summary of rotavirus biology relevant to vaccine production and control;
    - summary of status of vaccine development;
    - summary of key unresolved issues of relevance for vaccine regulation (no markers of attenuation, no correlates of protection, no models of intussusception, need for good post-marketing surveillance)

2. Scope
    - Vaccines that have been extensively studied in multiple countries
    - Additional guidance to be included for other vaccine candidates intended for international use

3. Product characterization (production and quality control specifications)
    - Control of vaccine source materials (based on existing WHO specifications)
    - Control of vaccine production (based on existing WHO specifications)
    - Summary protocol

3. Nonclinical evaluation of rotavirus vaccines
    - Reference to generic WHO guidelines
    - Need for toxicity evaluation after oral administration in animal studies
    - No need for neurovirulence test

4. Clinical evaluation of rotavirus vaccines
    - Reference to generic WHO guidelines
    - Need for reliance on post-marketing surveillance

It was agreed that a drafting group be constituted by WHO to develop the guidelines. This group would need to develop guidance on a number of unresolved issues that were identified during discussions. These included:

Quality issues

- how to assure comparability when a major change is made to a production process e.g. how to qualify a new working seed as attenuated?
- what is expected for TSE compliance of bovine derived vaccine strains?
- Whether, since one vaccine is lyophilised and another is a liquid product, a single thermostability test specification is applicable to all products ?
- whether there should be a requirement to test the thermostability of each strain in multivalent products?

Safety issues

- does there need to be an age restriction for age of first dose?
- how to distinguish between real and chance associations for rotavirus vaccine administration and intussusceptions?
- the transmissibility of vaccine strains to controls is unknown in low hygiene settings - does this matter?
- how to assure safety if major changes made to production process eg use of higher dose in vaccine; addition or subtraction of strains in multivalent vaccines?

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Efficacy issues

- will a single specification of virus potency be suitable in all hygiene settings?
- efficacy against wild-type virus strains not included in the vaccines unclear - does this matter?

Finally, the meeting considered international reference preparation needs and identified vaccine potency assay collaborative studies as a key first step. In the area of

coordinated research needs for quality, safety and efficacy of rotavirus vaccines the meeting gave a high priority to standardized post-marketing study protocols and recommended post-marketing capacity building, where necessary, in early introducer countries. The meeting also noted that studies were not yet completed in some key target populations, especially in those countries that will procure through UN agencies, and thus requested WHO to consider the implications for pre-qualification of rotavirus vaccines.

WHO Informal Consultation on Quality, Safety and Efficacy Specifications for Live Attenuated Rotavirus Vaccines

Mexico, D.F., Mexico
8-9 February 2005

Agenda

Tuesday 8 February

08:30 Opening remarks	Ma. de los Angeles Cortés, PAHO/AMRO
	David Wood, WHO
Self-introductions Election of chair and Rapporteur: Aims of the consultation	*Chair*

Session 1 The context

Overview of live attenuated rotavirus vaccine development.	*Roger Glass, CDC*
The role of WHO in developing global technical standards for quality, safety and efficacy of vaccines	*David Wood, WHO*
Regulatory evaluation of live attenuated rotavirus vaccines in the region of the Americas	Ma. de los Angeles Cortés PAHO/AMRO
Pre-qualification of vaccines by WHO	*Liliana Chocarro, WHO*

Discussion

Session 2 Status of Rotavirus Vaccine Development

Presentations from manufacturers who have licensed vaccines, or vaccine candidates in advanced development, on quality, safety and efficacy issues -

GSK Biologicals	*Corine Lecomte*
Merck Research Co	*Alan Shaw*

Session 3 Quality issues

Rotavirus biology relevant to evaluation of vaccine quality	*Harry Greenberg, Stanford University*
Control of vaccine source materials (cells, virus seeds and biologicals used in the manufacturing process)	*CD Atreya, FDA*
Control of vaccine production	*Maria Luz Pombo, NRA Venezuela*
Standardization of potency assays	*Philip Minor, NIBSC*
General discussion and wrap up of day 1	

Wednesday 9 February

Session 4 Safety issues

Rotavirus biology relevant to evaluation of vaccine safety	*Linda Saif, Ohio State University*
Non-clinical evaluation of vaccines - generic WHO Guidelines	*David Wood, WHO*
Specific non-clinical evaluation of rotavirus vaccines	*Georges Thiry, IAVI*
Safety evaluation of rotavirus vaccines in humans	*Timo Vesikari, Tampere, Finland*
Vaccine transmissibility surveillance	*Duncan Steele. WHO*

Session 5 Efficacy issues

Rotavirus biology relevant to evaluation of vaccine efficacy	*Manuel Franco, Univ. Javeriana Colombia*
Efficacy studies for rotavirus vaccines	*Irene Perez-Schael, Instituto de Biomedicina, Venezuela*

Standardization of rotavirus immune response assays	Richard L. Ward, Children's Hospital Medical Center, Cincinnati
Discussion	

Session 6 National control laboratory testing

Batch release tests for live attenuated rotavirus vaccines	*Roland Dobbelaer, NRA Belgium*
Discussion	

Session 7 Conclusions

Issues to be covered in WHO guidelines for quality, safety and efficacy of rotavirus vaccines
Identification of standardization needs
Identification for any research needed on quality, safety and efficacy of rotavirus vaccines

David Wood/WHO

Close of open part of meeting	Ma. de los Angeles Cortés, PAHO/AMRO

Session 8 (Closed session) Recommendations to WHO

National Regulatory Authorities

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