ROTAVIRUS VACCINES

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Abstract

Rotavirus, the most common cause of severe diarrhea and a leading cause of mortality in children, has been a priority target for vaccine development for the past several years. The first rotavirus vaccine licensed in the United States was withdrawn because of an association of the vaccine with intussusception. However, the need for a vaccine is greatest in the developing world, because the benefits of preventing deaths due to rotavirus disease are substantially greater than the risk of intussusception. Early vaccines were based on animal strains. More recently developed and licensed vaccines are either animal-human reassortants or are based on human strains. In India, two candidate vaccines are in the development process, but have not yet reached efficacy trials. Many challenges regarding vaccine efficacy and safety remain. In addition to completing clinical evaluations of vaccines in development in settings with the highest disease burden and virus diversity, there is also a need to consider alternative vaccine development strategies.

Key words: Enteric vaccine, rotavirus, vaccine

Since Edward Jenner’s experiments with cowpox and smallpox in 1796, scientists have been using methods to enhance the immune system and combat infectious diseases. Vaccination is now recognized as an effective public health policy for prevention of disease burden and mortality, provided the vaccines used are effective in terms of preventing disease in the majority of vaccinated individuals and also cost-effective.

Rotaviruses were described less than 40 years ago, but were quickly recognized as a major cause of diarrheal morbidity and mortality, particularly in children in developing countries. Rotavirus infection has a short incubation period of between one to three days. Rotavirus disease is characterized by the sudden onset of acute watery diarrhea, often accompanied by fever and vomiting. The virus infects and replicates almost exclusively in the mature enterocytes at the tips of the intestinal villi. It is believed that the destruction of the villi with the resultant loss of absorptive capacity of sodium, glucose and water is associated with the gastrointestinal symptoms of the infection. Rotavirus infection is often accompanied by serious fluid and electrolyte loss with dehydration, especially in infants. Furthermore, the rotavirus non-structural NSP4 protein, has been shown to act as a viral enterotoxin and induces diarrhea in the mouse model in an age-dependent and dose-dependent manner. The diarrhea is caused by excessive chloride secretion, possibly induced by a calcium-dependent signal transduction pathway.

Vaccine development efforts began in the early 1980s and continue until today. There are now two vaccines that have been licensed in developed countries after extensive safety trials and many vaccines in development in developing countries, including India.

The Rationale for Rotavirus Vaccines

A broad range of bacteria, viruses and parasites cause gastroenteritis in children, but the severe disease is most commonly associated with rotaviruses. A recent report from an Asian surveillance network showed that 45% of all diarrhea-related hospitalizations in children less than five years of age were attributable to rotavirus. Rotavirus infections occur in all children during the first few years of life, suggesting that the virus is not primarily transmitted through the oro-faecal route. Therefore, improved hygiene and sanitation alone cannot result in decreased rotavirus infections. Rotavirus infections frequently present with vomiting, which can result in discontinuation of oral rehydration therapy. Although first infections can lead to disease that ranges from mild gastroenteritis to severe or fatal diarrhea with dehydration, they also can induce immunity against severe disease after reinfection. Based on these data, vaccines have been identified as the best current strategy to decrease the burden associated with severe and fatal rotavirus diarrhoea.

During the past two decades, discussion among many groups, including the World Health Organization (WHO), the Institute of Medicine and the Global Alliance for Vaccines and Immunization (GAVI), have identified rotavirus vaccines as a priority for development. This decision has been based primarily on the enormous toll of rotavirus disease. It is estimated that each year, 440,000 children under five years of age die of rotavirus gastroenteritis, two million are hospitalized and 25 million require an outpatient visit. In poor countries, approximately one child in every 250 will die of rotavirus disease by five years of age.
Recent hospital-based surveillance performed in Asia indicates that 20 to 50% of hospitalizations for diarrhea among children less than five years of age are associated with rotavirus infection and the morbidity and mortality may be higher than previously estimated.12,13 Even in the developed world, vaccines could prevent severe rotavirus disease that, in the United States alone, is associated with 500,000 physician visits, 60,000 hospitalizations, 20-40 deaths and annual costs exceeding $1 billion.14

Rotavirus Diversity and Implications for Vaccine Development

Rotaviruses are classified into seven different serogroups (A-G) based on the antigenic specificity of the capsid proteins of the virus, as well as the pattern of electrophoretic mobility of the 11 RNA segments of the viral genome.15 The inner capsid protein VP6 comprises the greatest mass of the particle and this is the protein used more frequently to detect and distinguish viruses into different groups. Of the seven serogroups, only Groups A, B and C are known to infect humans and Group A viruses are those that cause severe, life-threatening disease in children worldwide. For Group A viruses, further typing schemes were introduced based on antigenic epitopes on the proteins that form the inner capsid (VP6, subgroups I and II) and on proteins of the outer capsid, the glycoprotein VP7 (G serotypes) and the spike protein VP4 (P serotypes). VP7 and VP4 elicit neutralizing antibodies. Neutralizing mouse monoclonal antibodies for typing VP7 are easily derived and have been used extensively in epidemiological surveys.16 In recent years, reverse-transcription polymerase chain reaction has been used in molecular epidemiological studies.17 All known G serotypes have been correlated with genotypes; however, there are more P genotypes than serotypes identified leading to a serotype/genotype dual nomenclature for P types.18

The intensive epidemiological studies over the last 30 years since rotavirus was first described, have shown that it is the most common cause of life-threatening gastroenteritis in children. The incidence and distribution of group A rotavirus serotypes and genotypes vary between geographical areas during a rotavirus season and from one season to the next. Globally, G1 to G4 and P1A[8] and P1B[4] are the most common in G- and P- types causing disease in humans. The application of reverse-transcription polymerase chain reaction genotyping and nucleotide sequencing has helped in the identification of strain diversity, with at least 42 P-G combinations being recognized in human infections and has also helped in the identification of additional globally (G9) and regionally (G5, G8, P 2A[6]) common strains that are not covered by the vaccines that have undergone trials.18

Rotaviruses diversify and evolve mainly through two mechanisms. The first is the accumulation of point mutations, which generates genetic lineages, and leads to the emergence of antibody escape mutants. The second mechanism is genetic shift, where exchange of genetic material through gene reassortment occurs during dual infection of a single cell.19 Also, zoonotic transmission and gene re-assortment between human and animal rotaviruses contributes to the generation of diversity of rotaviruses infecting humans.20 The diversity and capacity of human rotaviruses for rapid evolution and re-assortment suggest that rotavirus vaccines must provide good heterotypic protection to be effective in different geographic regions.

Early Vaccines

Immunity to rotavirus infection in infants was first demonstrated by Bishop et al.,9 who observed that newborns who were infected with rotavirus were protected against severe diarrhea after re-infection. Rotavirus vaccine development has mainly focused on the delivery of live attenuated rotavirus strains by the oral route. The initial “Jennerian” approach involving bovine animal rotavirus vaccine candidates, based on the premise that these would be naturally attenuated outside their normal host, showed that these vaccines were safe, well tolerated and immunogenic (Table 1). The first rotavirus vaccine trials, in which the bovine strain RIT4237 (P6[1], G6) was used, yielded an efficacy of 55-62% against any rotavirus diarrhea and 80-88% efficacy against more severe disease.20 Despite this encouraging start, both RIT4237 and another bovine strain, WC3 (P7[5], G6), later produced inconsistent results. When tested in developing countries, poor efficacy was observed in trials conducted in developing countries including the Gambia, Peru, Rwanda and Central African Republic.21 A rhesus strain vaccine (RRV) (PSB[3], G3), was tested extensively, including in a relatively poorer country, Venezuela, where it had 85-90% efficacy against the most severe disease.22

Monovalent animal strain vaccines have been mostly abandoned, with the exception of the Lanzhou lamb rotavirus vaccine (LLR). LLR, a P[12],G10 strain, is a monovalent, ovine strain vaccine produced by the Lanzhou Institute and licensed in China in 2000. Trials indicated that 6 to 24-month-old children given a single dose of vaccine mounted good immune response to the vaccine, with the vaccine showing variable efficacy in developing countries.

<table>
<thead>
<tr>
<th>Vaccine strain</th>
<th>Type</th>
<th>Developer</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIT 4237</td>
<td>Bovine monovalent</td>
<td>Smith Kline (1983)</td>
<td>Poor efficacy in developing countries</td>
</tr>
<tr>
<td>RIT 4256</td>
<td>Bovine monovalent</td>
<td>Smith Kline (1986)</td>
<td>Immunogenicity similar to RIT 4237</td>
</tr>
<tr>
<td>WC3</td>
<td>Bovine monovalent</td>
<td>Wistar Institute (1984)</td>
<td>Variable efficacy in developing countries</td>
</tr>
<tr>
<td>RRV</td>
<td>Rhesus monovalent</td>
<td>National Institutes of Health (1985)</td>
<td>Variable efficacy in developing countries</td>
</tr>
</tbody>
</table>
responses consistent with booster responses and had no associated adverse events. 10 Although the vaccine is available in some parts of China, it is not included in national immunization programs in China or elsewhere.

Human-Animal Reassortant Vaccines

The early studies led to the concept that a multivalent vaccine that represented each of the four epidemiologically important VP7 serotypes might be necessary to induce protection in young infants, the target population for vaccination. Human-animal rotavirus reassortants whose gene(s) encoding VP7, with or without VP4, were derived from their human rotavirus parent but whose remaining genes were derived from the animal rotavirus parent were developed as vaccine candidates. These reassortants induced immune responses to the human capsid proteins, 23 while maintaining the attenuation properties of the parent strain. Several reassortant vaccines have been produced and tested, based on either simian or bovine strains.

Quadrivalent RRV-based rhesus-human reassortant vaccine

Rotashield®, now licenced to BioVirx, USA, is a quadrivalent human-rhesus reassortant vaccine that includes RRV, which is a G3 strain and 3 reassortants representing G1, G2 and G4 strains. 24 Multiple large efficacy trials were conducted and the vaccine elicited 50-60% protection against all rotavirus diarrhea and 70-100% protection against severe rotavirus disease. Rotashield® was associated with a short duration of fever after the first vaccination, but pre-licensure, no other adverse events were commonly associated with vaccine. 24

This vaccine was the first rotavirus vaccine in the world and was licensed in the USA in 1998 and recommended by the Immunization Practices Advisory Committee and the American Academy of Pediatrics for inclusion in the U.S. childhood vaccine schedule. 24 Recommendations for use of the vaccine were suspended in 1999 after reports of intussusceptions among vaccine recipients. 25 After investigations demonstrated a significant association between receipt of the vaccine and occurrence of intussusception, 26 the vaccine was withdrawn.

Pentavalent WC3-based bovine-human reassortant vaccine

Like RRV, the WC3 vaccine strain was developed into a series of human-bovine reassortant vaccine strains which were then combined into a polyvalent vaccine. 27 Compared with the rhesus reassortants, the bovine-human reassortants appear to cause less fever while maintaining immunogenicity. 28 RotatetM, manufactured by Merck, Inc, USA, is a pentavalent vaccine containing five reassortants representing the common human VP7 types, G1-4 and the most common VP4 type, P[8]. A large efficacy trial with RotatetM has been completed, which found 74 and 98% efficacy against all and severe disease, respectively and has efficacy against each of the common circulating serotypes. A large safety trial that with more than 70,000 infants, half of whom received vaccine, found no evidence of an increased risk of intussusceptions among vaccinees compared with placebo recipients. 7 Merck has received recently licensure in the United States and the vaccine has been recommended by the ACIP for inclusion in routine immunization of infants in the United States.

U.K.-based bovine-human reassortant vaccine, National Institutes of Health, Bethesda, MD

A second bovine-based reassortant vaccine has been developed and tested based on the U.K. rotavirus, a P[7], G6 strain. Reassortants representing common human VP7 serotypes have been produced. In a U.S. trial, the vaccine demonstrated satisfactory levels of attenuation, safety, infectivity and immunogenicity of each monovalent reassortant in infants. 29 In a trial in Finland, the vaccine provided >90% protection against severe rotavirus disease. Its development has been taken over by manufacturers in Brazil, India and China.

Human Rotavirus Strain Vaccines

Human rotavirus strains, which have been developed as vaccines or vaccine candidates are either attenuated common strains or uncommon strains isolated from asymptomatic neonates.

Monovalent human G1 rotavirus vaccine

Rotarix®, developed by GlaxoSmithKline Biologicals, Belgium, is a monovalent, P1A[8], G1 rotavirus derived from a human G1 strain (89-12) that yielded high efficacy in early trials in the US and Finland. 30 The vaccine has been tested in Latin America; the first results from these multi-country trials were reported from Mexico, Brazil and Venezuela where efficacy against severe rotavirus disease was 86%. 31 Efficacy has been confirmed in a series of trials in which >63,000 children were enrolled. In these studies, efficacy against hospitalization was 85% and efficacy against non-G1 serotypes was 75%. 31 In these trials, no significant adverse events or increased risk of intussusceptions was observed among vaccinees. The vaccine was first licensed in Mexico and the Dominican Republic in 2004 and licensure is being sought in additional countries. Data from trials in developing country settings are expected in the next few years.

RV3 neonatal strain vaccine

RV3, a P2A[6], G3 strain, was first isolated from newborns at the Children’s Hospital in Melbourne, Australia. 32 This vaccine is based on an observation that neonates infected with this rotavirus strain in hospital nurseries usually were asymptomatic and were later protected against severe disease in early childhood. Initial safety trials in which a single dose of vaccine was used demonstrated no significant adverse events, but serum immune responses were poor. 33 A trial of
three doses of vaccine-induced immune responses in 54% of infants and vaccinees that developed an immune response were protected from rotavirus disease. Because of the promising results, the developers are working with BioPharma (Bandung, Indonesia) to increase the titer of this vaccine and return to clinical trials.

Indian neonatal strain vaccines

Two strains isolated from newborns in India are being prepared as candidate vaccines. Strain 116E, isolated in 1985 from an ‘outbreak’ of asymptomatic rotavirus infections in New Delhi, is a P8[11], G9, natural reassortant between a human parent strain and the VP4 gene of bovine origin. The sequence of the VP4 gene was homologous to P[11], a genotype commonly found in cattle. A nosocomial outbreak of infection at a maternity center in Bangalore led to the identification among neonates of another “outbreak” due to strain I321 that was also a bovine-human reassortant strain. Unlike strain 116E, strain I321 had a base of nine bovine gene segments and only gene segments five and seven, which encoded nonstructural proteins one and three, were of human origin. A strain with the same G and P characteristics as strain I321 has emerged as a cause of diarrhea in children in Vellore, India.

These two Indian neonatal strains have interesting differences in their genomic structure. Strain 116E is a human strain with a single bovine VP4 gene, while the I321 strain is composed primarily of bovine genes and has only two segments that are of human origin. Both strains are in preclinical development and human trials are being planned in India, but with the new finding of I321-like strains causing disease in children, both careful epidemiological studies and safety monitoring will be essential prior to licensure.

### Other Approaches to Rotavirus Vaccination

The actual immunological mechanism by which protection against rotavirus disease, after natural infection or after immunization, occurs is unknown. Rotavirus infection results in both serum and intestinal antibody and protects against severe diarrheal illness upon subsequent infection. This difficulty in understanding the mechanism of protection has made interpretation of various clinical trials difficult where variable efficacy results were obtained. In brief, most studies on immune responses have indicated that the presence of faecal IgA or serum antibodies serves as a good surrogate marker for protection, although it is believed that other effector mechanisms of the immune response are important. These are undefined in humans at the moment, although animal studies point to the importance of CD4 and CD8 T cells.

Although live virus vaccines orally administered, represent the primary approach to rotavirus vaccine development, other approaches and routes of administration are being evaluated and tested in animal models. Work on virus-like particles, cold-adapted strains, inactivated strains and DNA vaccines (Table 2) have been undertaken. These approaches could have some advantages in the future if they could improve the variable immune response to oral vaccines, could be combined with other parenterally administered vaccines or avoid intussusceptions.

### Challenges for Rotavirus Vaccine Development and Introduction

Introduction of a new vaccine faces many hurdles, including cost, production capabilities and programmatic issues. For rotavirus vaccines, while there is clearly a need,
there are also additional challenges based on the nature of the disease and the safety issues raised by the association of the previous vaccine with intussusceptions.

The attributable risk of vaccine-associated intussusceptions has been estimated in different studies to be ~1 child in 10,000,41 to ~1 in 18,000 -302,000 Rotashield® recipients.42 If such low risks exist with new rotavirus vaccines, they will be uncovered only with adequate and extensive surveillance after vaccine introduction.

The observation that previous rotavirus vaccines generally have yielded poor efficacy when tested in developing countries has led to concerns about the potential effectiveness of any future live oral rotavirus vaccine in these settings.43 Although rotavirus infection is universal early in childhood, the epidemiology of the disease is quite different in developed and developing countries. Differences in age of first infection, strain distribution, occurrence of mixed infections, seasonality and risk of mortality can affect decisions about vaccine composition and delivery. Higher doses of vaccine or additional doses may be required to overcome the inhibitory effects of competing intestinal flora, concomitant use of oral polio vaccine and high levels of humorally transferred maternal antibodies against rotavirus. Despite a recommendation by the WHO that all rotavirus vaccines be tested early in developing countries, no data from the current vaccines are available in Africa or poor Asian countries,40 although testing of Rotarix is under way in Bangladesh and South Africa. These data will be critical to determining the probability of success of the current rotavirus vaccines and to establish requirements for vaccines in preclinical and clinical development.

References


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