Review Article

A Rotavirus Vaccine for Infants: The Asian Experience

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Abstract

Introduction: Severe rotavirus gastroenteritis in children causes significant morbidity worldwide and substantial deaths in developing countries. Hence, a live attenuated vaccine Rotarix™ was developed with human strain RIX4414 of G1P1A P[8] specificity. RIX4414 trials in infants have begun in developed and developing countries worldwide. An overview of RIX4414 in developed and developing countries and prospects with this vaccine in Asia are presented. Methods: Completed RIX4414 trials have been reviewed. Results: Two oral doses of RIX4414 were well tolerated with a reactogenicity profile similar to placebo. RIX4414 was also highly immunogenic, e.g., in a dose-ranging study conducted in Singapore, 98.8% to100% of infants had a vaccine take after 2 doses. RIX4414 did not affect the immune response of simultaneously administered routine infant vaccines. RIX4414 significantly reduced severe rotavirus gastroenteritis in settings where multiple serotypes including the emerging G9 type co-circulated. Conclusion: These encouraging results warrant further evaluation of the vaccine worldwide and especially in developing countries with the highest need. Therefore, evaluation of the Rotarix™ vaccine is continuing in large phase III trials in Asia and worldwide.

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Introduction

Of all the enteric pathogens that infect young children, rotavirus is recognised as the leading cause of severe gastroenteritis worldwide. Rotavirus accounts for 20% of all diarrhoea-related deaths and global mortality among children less than 5 years of age is estimated at nearly half a million.[1] Rotavirus mortality is concentrated in the developing countries in the Asian subcontinent, Africa and Latin America. Rotavirus is estimated to cause death in 1 of every 111 to 203 Bangladeshi children[2] and up to a staggering 100,000 deaths in India[3] every year. The deaths due to severe rotavirus gastroenteritis occur from ensuing dehydration in the impoverished developing areas, where access to health care facilities is limited. Moderate to severe rotavirus gastroenteritis is estimated to cause over 2 million hospitalisations and 25 million clinic visits among children less than 5 years of age each year worldwide.[1] Although hospitalisation rates vary, rotavirus is an important cause of hospitalisation in developed and developing countries in Asia.[2,4,5] It should be noted that in rural developing settings, hospitalisation rates only represent children who were able to travel for care and may underestimate severe disease rates. Medical costs associated with treatment or hospital stays and indirect societal costs also contribute to the global rotavirus burden, a burden that is especially evident in highly industrialised countries, reaching over US$1 billion annually in the United States.[6]

Rotavirus infection during early childhood is practically inevitable, though the time of its occurrence may vary depending on the presence or absence of a seasonal peak

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determined by the region's climate and latitude. Seasonality is less marked in tropical countries and therefore exposure to rotavirus occurs throughout the year.[7] Several studies have shown that early rotavirus infections impart protective immunity in children.[8-12] More importantly, a study in Mexican children demonstrated that a single rotavirus infection provided excellent protection against subsequent severe rotavirus disease, and 2 previous infections provided complete protection against both moderate to severe rotavirus gastroenteritis.[9]

An important feature of rotavirus is the diversity of circulating strains belonging to different serotypes as determined by the outer capsid VP7 (G type) and VP4 (P type) proteins. Until recently, G serotypes 1 to 4 associated with P[8] and P[4] genotypes were the major circulating rotavirus strains. G1P[8] has been the predominant strain, followed by G3P[8], G2P[4] and G4P[8] in most countries.[13] A fifth G serotype, G9, has been found in geographically distant countries such as Australia, the Indian subcontinent, the United Kingdom, Latin America and the United States[14-20] and its prevalence has been steadily increasing. For example, a hospital-based surveillance study conducted in 6 Indian cities found the G9 serotype in 17% of children hospitalised for rotavirus diarrhoea between 1996 and 1998,[15] a substantial increase from 1993 when the G9 type accounted for 9.5% of rotavirus cases.[21] Mixed infections with rotaviruses of different G and P types are common in developing countries.[15,16,22] Unusual G serotypes (G5, G8 and G10) and common G serotypes in association with unusual genotypes (P[6] and P[9]) have also been reported, particularly in developing nations where the diversity of circulating rotavirus strains is greatest.[23-25] Natural infection with one rotavirus serotype clearly induces protection against different serotypes,[9] indicating that protection is heterotypic. On this basis, an attenuated human strain is expected to provide cross-protection against different rotavirus strains. The factors responsible for heterotypic protection have not yet been fully determined but both the presence of cross reacting epitopes on the outer capsid neutralisation proteins, VP4 and VP7,[26] and serotype-independent T cell responses to epitopes on any of several rotavirus proteins are likely to play important roles.

Rotavirus is a major public health problem due to its associated morbidity and mortality. Thus, there is a clear need for an effective intervention that can prevent severe rotavirus illness among young children worldwide, especially in developing countries where the death toll due to rotavirus disease is high.

Is there a Need for a Rotavirus Vaccine?

Fluid and electrolyte replacement by oral or intravenous routes to treat dehydration resulting from severe rotavirus gastroenteritis saves lives but does not reduce morbidity nor the spread of the virus. Besides, adequate treatment depends on access to a health care facility during severe episodes. This is especially problematic in rural areas of developing nations where untreated dehydration often leads to death. Improved sanitation or hygiene measures generally decrease the incidence of diarrhoeal diseases of bacterial origin but in the case of rotavirus, which is transmitted mainly by the faecal-oral route, there is no appreciable difference in the incidence of disease between developed and developing countries. Essentially, rotavirus knows no boundaries, and all young children are equally vulnerable.

An effective intervention to prevent severe rotavirus gastroenteritis, such as vaccination, is a global need. In fact, the development of an effective rotavirus vaccine has been and continues to be recognised as a priority by the World Health Organization.[27] The immunising effect of natural infection as witnessed by declining incidence of rotavirus diarrhoea with age and protection against subsequent rotavirus illnesses[9] supports immunisation against rotavirus early in life as an effective preventive measure. The main goal of a rotavirus vaccine should be to prevent severe rotavirus gastroenteritis that can lead to dehydration, hospitalisation and/or death. If this vaccine were composed of a live virus, the goal would be to reproduce the protection against severe rotavirus gastroenteritis as seen following natural infection but without the associated illness.

Rotavirus Vaccine Development

Efforts to develop rotavirus vaccines began soon after the human strain was discovered, but more than 3 decades later, there is still no rotavirus vaccine available for universal use. The first bovine-derived rotavirus vaccine candidates were generally efficacious in industrialised settings but were less effective in developing countries.[27-32] Except for a vaccine based on a lamb strain[33] locally produced and only licensed in China, the tetravalent rhesus-human reassortant vaccine (RotaShield™, Wyeth Laboratories) was the only vaccine licensed and used in the USA. Rotashield™ was evaluated mainly in industrialised countries and was proven effective.[34] In an efficacy trial conducted in Venezuela,[35] protection was comparable to that found in Europe and North America. However, this was not seen or found in Brazil and Peru where efficacies were very low.[32,36] A study in Bangladesh showed that RotaShield™ was safe and immunogenic,[37] but the vaccine was not evaluated for efficacy because of its association with intussusception, which has led to its withdrawal from the United States market.[38] A lack of large and more comprehensive data in developing areas in Asia, Africa and Latin America prevented the evaluation of risk-benefit ratios for intussusception versus prevention of severe

rotavirus disease that can lead to hospitalisation and death.

The search for safer vaccines continued and 2 promising candidates were the live quadrivalent human-bovine rotavirus vaccine developed by Merck and Co[39] and the human rotavirus (HRV) vaccine strain 89-12 developed by investigators at Cincinnati Children's Hospital and Avant Immunotherapeutics.[40] The quadrivalent human-bovine rotavirus vaccine was generally well-tolerated, with no differences between vaccine and placebo recipients in the incidences of fever, irritability, vomiting or diarrhoea during the 14 days after any dose. The vaccine was immunogenic and 75% (95% CI, 49% to 88%) efficacious in preventing any rotavirus acute gastroenteritis. The HRV vaccine was developed by attenuating the virulent wild type 89-12 strain (G1P1A P[8] specificity) by multiple passages in cell culture. During a two-year trial in the United States, the 89-12 vaccine at a virus concentration of 10^5 foci forming units (ffu) showed 76% (95% CI, 54% to 87%) efficacy in young children against any rotavirus gastroenteritis and 84% (95% CI, 57% to 94%) efficacy against severe rotavirus gastroenteritis (defined as >8 points on a 20-point scale[41]).[42] The 89-12 vaccine was immunogenic and the only side effect seen in vaccinees relative to placebo recipients was increased mild fever. A new human rotavirus vaccine (RIX4414, Rotarix™) containing the next generation of the 89-12 vaccine strain was subsequently developed for further clinical evaluation.

Rotavirus Vaccine: RIX4414 (Rotarix™)

The live attenuated human rotavirus vaccine, RIX4414 (Rotarix™) was developed from the parent 89-12 vaccine strain by GlaxoSmithKline Biologicals, Rixensart, Belgium. The vaccine was to be given orally after reconstitution with a liquid calcium carbonate buffer.

After the safety of RIX4414 was verified in healthy adults and toddlers in Europe,[43] clinical evaluation of this new vaccine candidate was initiated in healthy infants not previously infected with rotavirus in clinical studies in Europe,[43,44] Latin America,[45] Asia[46] and South Africa.[47] During randomised, double-blind and placebo controlled trials, 2 oral doses containing $10^{4.7}$ up to $10^{6.1}$ ffu of RIX4414 per dose were tested in infants. The immunisation schedule followed the national recommendations for routine infant immunisations and, therefore, vaccine doses were separated by 1 or 2 months. The placebo was identical to the vaccine except that it did not contain the rotavirus RIX4414 strain.

Evaluation of Rotarix™ in Asia began with a trial in Singapore.[46] The trial was conducted at paediatric hospitals and polyclinics in Singapore and involved 2464 infants between 11 and 17 weeks of age at the time of the first dose. Three concentrations ($10^{4.7}$, $10^{5.2}$ or $10^{6.1}$ ffu) of RIX4414 were tested versus placebo. The first 2 doses of the routine infant vaccinations [diphtheria, tetanus, pertussis, polio, *Haemophilus influenzae* type b (DTPa-IPV/Hib) (Infanrix™-IPV, Hiberix™)], were administered concomitantly with RIX4414 or placebo according to the local schedule at 3, 4 and 5 months of age. Hepatitis B vaccine (Engerix™) was given at birth and 1 and 5 or 6 months of age.

Information on specific adverse events occurring 15 days after each dose was recorded by parents or guardians of vaccinated infants on diary cards provided on the day of the first vaccination. Two doses of Rotarix™ given simultaneously with routine vaccinations were well tolerated in Singaporean infants. The incidence rates for adverse events were similar between vaccine and placebo groups. RIX4414 did not cause increased diarrhoea, vomiting, fever (rectal temperature ≥38ºC), irritability or decreased

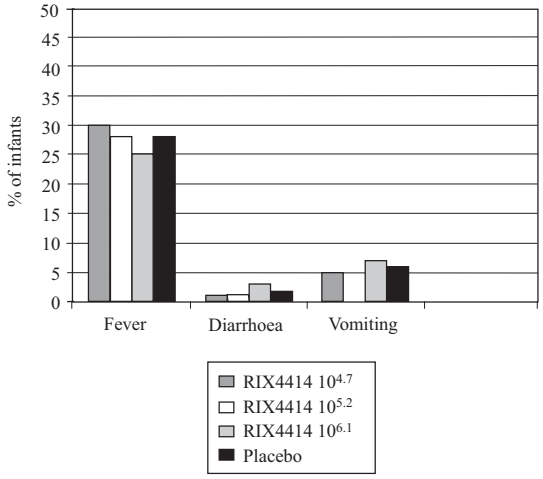

Fig. 1a. Percentage of infants reporting fever, diarrhoea and vomiting within 15 days after dose 1 of Rotarix vaccine in Singapore.

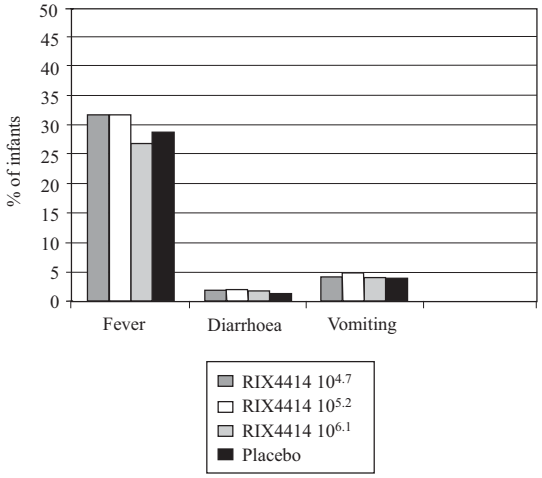

Fig. 1b. Percentage of infants reporting fever, diarrhoea and vomiting within 15 days after dose 2 of Rotarix vaccine in Singapore.

appetite compared to the placebo. Figures 1a and 1b illustrate the incidences of diarrhoea, fever and vomiting. There was no increase in clinically significant reactions such as high fever (axillary temperature >39ºC), severe diarrhoea (≥6 looser than normal stools/day) or vomiting (≥3 episodes of vomiting/day) after either dose or with increased viral concentration.[46]

Serious adverse events that occurred during the study were to be reported to the sponsor irrespective of causal relationship to vaccination. Special attention was focused on monitoring intussusception cases. All serious adverse events were reviewed periodically by an Independent Committee consisting of clinical experts and a statistician. Procedures for follow-up and work-up of any intussusception cases were also specified. Two infants were hospitalised due to vaccination-related fever after dose 1, and 2 intussusception cases were identified, one in temporal association (onset 6th day after dose 1 of vaccine) and one which occurred remotely (onset 10 months after dose 2 of placebo). All 4 children recovered promptly without sequelae. Laboratory data could not confirm or dismiss association of the intussusception case with vaccination and no conclusion can therefore be reached.[46] Observing 1 case in 2464 infants in the first year of life was in line with the intussusception background incidence of 66 or 41 or 32 per 100,000 in under 1-year-olds reported for the years 2000 to 2002, respectively.[48] Overall, for Rotarix™ phase I and II trials involving more than 7000 vaccinated infants, the intussusception incidence rate in the vaccine group and the placebo group was 0.06% and 0.05%, respectively.

The immunogenicity of the RIX4414 was primarily evaluated by measuring serum rotavirus-specific IgA using ELISA (assay cut-off: 20 units/mL) after vaccination. The vaccine was highly immunogenic in Singaporean infants and most vaccinees seroconverted (Table 1). Seroconversion rate was defined as the percentage of infants with a post-vaccination anti-rotavirus IgA antibody concentration of ≥20 units/mL among those who were negative for rotavirus IgA (i.e., <20 units/mL) before the first dose. RIX4414 stool shedding was also detected by ELISA in a large proportion of vaccinated infants, typically on the 7th day after dose 1 (Fig. 2). Subsequently, shedding waned steadily over time. A combined endpoint for vaccine response based on serum rotavirus IgA seroconversion and/or RIX4414 shedding in post-vaccination stools was defined as vaccine take. Virtually all infants in Singapore had vaccine take after 2 doses at all 3 dosage levels (Table 2). Some infants excreted the vaccine virus in detectable titres without demonstrating a measurable IgA antibody response. Therefore, vaccine take, rather than rotavirus IgA seroconversion alone, appears to be a more complete marker for a response to the vaccine. Overall, RIX4414 concentration of $10^{5.2}$ ffu or higher showed enhanced seroconversion rates. The majority of Singaporean infants seroconverted already after the first dose with little increase in the seroconversion rate after the second dose. However, because shedding of rotavirus was detected in >10% of vaccinees after dose 2 in infants with no evidence of vaccine take after dose 1, it appears that 2 doses are needed to maximise vaccine take.

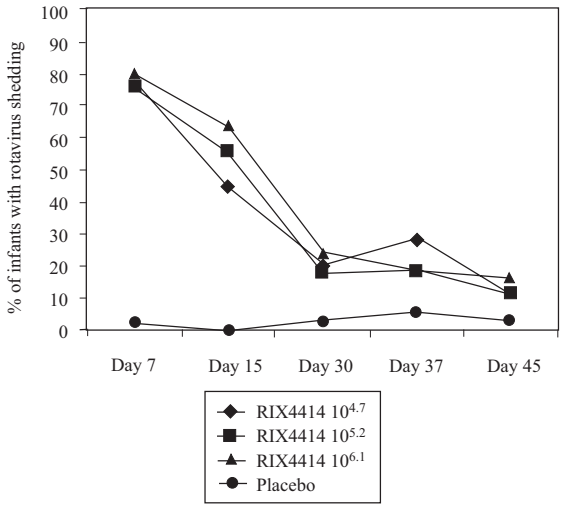

Fig. 2. Rotavirus shedding after each dose of Rotarix vaccine among infants in a study in Singapore.

Table 1. Rotavirus Specific Serum IgA Response after Each Dose of Rotarix Vaccine Among Infants in a Study in Singapore

	After dose 1			After dose 2		
RIX4414 concentration	n	Seroconversion rate (95% CI)	GMC (95% CI)	n	Seroconversion rate (95% CI)	GMC (95% CI)
$10^{4.7}$ ffu	142	74.6 (66.7-81.6)	282.2 (225.2-353.6)	146	76.0 (68.3-82.7)	272.5 (220.6-336.6)
$10^{5.2}$ ffu	147	86.4 (79.8-91.5)	328.7 (265.9-406.3)	145	91.0 (85.2-95.1)	298.0 (250.4-354.7)
$10^{6.1}$ ffu	153	81.0 (73.9-86.9)	327.8 (270.1-397.8)	154	88.3 (82.2-92.9)	249.3 (205.5-302.6)

95% CI: 95% confidence interval; GMC: geometric mean concentration; n: number of infants with available results
Blood samples were drawn 1 month after each dose.
Seroconversion rate defined as percentages of infants with post-rotavirus-specific IgA antibody concentration ≥20 units per milliliter in infants who were negative for rotavirus prior to the first dose of Rotarix™ vaccine or placebo.
Geometric mean concentrations are calculated for infants who seroconverted.

Table 2. Vaccine Take after Two Doses of Rotarix Vaccine among Infants in a Study in Singapore

RIX4414 concentration	n	Vaccine take (95% CI) after 2 doses
$10^{4.7}$ ffu	35	100 (90.0-100)
$10^{5.2}$ ffu	47	97.9 (88.7-100)
$10^{6.1}$ ffu	46	97.8 (88.5-100)

95% CI: 95% confidence interval; n: number of infants with available results

Vaccine take after 2 doses defined as seroconversion after either dose and/or vaccine rotavirus shedding in any stool sample collected from the day of dose 1 until post-vaccination blood sampling after dose.

There was no observed interference when Rotarix™ was co-administered with routine childhood vaccinations against diphtheria, tetanus, pertussis, polio, and *H. influenzae* type b.[46] It should also be noted that there was no interference with a hepatitis B vaccine which was co-administered in a subsequent Latin American study but was given separately at months 0, 1 and 5 or 6 in the Singapore study. While the latter study has evaluated simultaneous administration of inactivated poliovirus vaccine, other studies specifically evaluating simultaneous administration of Rotarix™ and oral polio vaccine are being conducted in South Africa[47] and Latin America. First results show no interference after full immunisation schedule for polio.[47]

Asia as Part of a Worldwide Experience

Other studies conducted with the new attenuated human rotavirus vaccine, Rotarix™, have shown that this vaccine is consistently well tolerated and immunogenic in different settings. IgA seroconversion rates in Singapore were comparable to results found in Finland[44] but were higher than those found in a trial conducted in Latin America (Brazil, Mexico and Venezuela).[45] Although it is not possible to directly correlate immune response to protection since serologic markers of protection are lacking, good protection can be expected on the basis of the excellent vaccine take found in the Singapore trial.[46] In the trials conducted in Finland and Latin America with different viral concentrations, RIX4414 showed significant protection against severe (defined as ≥11 points on a 20-point scale[49]) rotavirus gastroenteritis [66% (95% CI, 32% to 84%) to 90% (95% CI, 10% to 100%) efficacy] and significantly reduced rotavirus-related hospitalisations [65% (95% CI, -2% to 90%) to 93% (95% CI, 54% to 100%) efficacy][45] depending on the vaccine virus concentration and the setting. Importantly, RIX4414 induced significant clinical protection against severe disease caused by non-G1 (primarily G9) strains [65% (95% CI, 7% to 89%) to 83% (95% CI, 40% to 97%) efficacy depending on the vaccine virus concentration] in Latin America,[45] where multiple heterotypic strains were circulating.

The clinical proof of both homotypic and heterotypic protection was observed in the Latin America study and this concept needs to be further evaluated through field testing in different settings, including areas where vast diversity in circulating strains is reported. In conjunction with vaccine evaluations, national epidemiological surveillance of rotavirus is crucial for developing and developed countries due to the diversity of circulating strains and differences in morbidity/mortality rates in different countries within a region. Current disease burden estimates obtained from regional surveillances such as the multi-country hospital-based rotavirus surveillance conducted by the Asian Rotavirus Surveillance Network[50] will be crucial to assess vaccine need and the associated public health value. The significant impact of rotavirus vaccination on severe disease rates will result in substantial savings in direct and indirect costs. Current economic burden data are, however, lacking for several countries. Surveillance for health-economics estimations in different settings is necessary since cost-effectiveness of vaccination programmes will play an important role in national policymaking.

The association of intussusception with the rhesus-based vaccine has changed the evaluation for safety of rotavirus vaccines and probably other new live viral vaccine candidates as well. Rotarix™ has exhibited a consistently mild reactogenicity profile similar to the placebo. Fever and diarrhoea are not associated with this vaccine and the overall reactogenicity profile is superior compared to the data published on RotaShield™ that was associated with increased post-vaccination fever.[51] Rotarix™ has to date been found to be safe in 70,000 infants and the intussusception rates observed have been similar between vaccine and placebo groups. Indeed, in addition to the 2 cases reported in Singapore, no case of intussusception was reported in Europe. One case was reported in Latin America (Brazil, Mexico, Venezuela) remotely from vaccination (6 months after the second dose of $10^{4.7}$ ffu).[52] First results of a large multi-country trial in Latin-America and Finland involving over 63,000 infants indicated that in the 31 days-window after each dose, 6 and 7 cases were respectively observed in the vaccine and placebo groups.[53] All children completely recovered. The overall incidence of intussusception across all these studies was 0.02% in the vaccinees and in the placebo groups. These rates are in line with reported background rates of 0.04% for infants under 1 year of age.[54] Administration of RIX4414 mimics natural infection, which makes it a potentially safer vaccine candidate. Natural infection with wild type rotavirus is not expected to be associated with intussusception as seen from a lack of intussusception peaks during the winter rotavirus epidemics.[4,55] Moreover, attenuation should make RIX4414 rather less likely to be associated with

intussusception. Whether intussusception was a side-effect specific to the rhesus vaccine can be determined only by conducting large prospective cohort trials with the newer rotavirus vaccine candidates.

The encouraging clinical results with Rotarix™ are a first step in making a safe vaccine available for effective control of infant rotavirus gastroenteritis globally. A safe and effective rotavirus vaccine that can prevent severe disease and deaths with 2 doses given in early infancy with other routine immunisations seems possible in the near future. To reach this goal globally, evaluation of the vaccine is continuing worldwide and especially in areas where it is urgently needed.

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