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RIX4414 (Rotarix™): a live attenuated human rotavirus vaccine

David I. Bernstein*

Rotaviruses are recognized as the single most important cause of severe infantile gastroenteritis worldwide. On a world scale, rotaviruses are estimated to be responsible for over 600,000 deaths annually.[1,2] For these reasons, rotaviruses have received a high priority as a target for vaccine development.[3] Incorporation of an effective rotavirus vaccine into the infant immunization schedule in developed countries could reduce hospitalizations due to dehydrating diarrhea in young children by 40 to 60%.[4] More important, the worldwide use of such vaccine could decrease the total number of deaths caused by diarrhea by approximately 10 to 20%.[1,4]

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Transmission of rotaviruses occurs by the fecal-oral route, providing a highly efficient mechanism for universal exposure that has circumvented regional and national cultural practice differences. The symptoms associated with rotavirus disease typically are diarrhea and vomiting accompanied by fever, nausea, anorexia, cramping, and malaise that can be mild and of short duration or produce severe dehydration.[5,6] Severe disease occurs primarily in young children, most commonly among those aged 6 to 24 months. Approximately 90% of children in both developed and developing countries experience a rotavirus infection by the time they reach 3 years of age.[5]

Initial efforts to develop a rotavirus vaccine relied largely on the use of a single animal strain to create a live attenuated oral vaccine that would provide protection from severe disease. Because efficacy results were inconsistent, these attempts were modified to include multi-component

* MD, MA. Professor of Pediatrics, Albert Sabin Education Center, Cincinnati Children's Hospital Medical Center, University of Cincinnati, Cincinnati, OH, USA.

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human-animal reassortant vaccines, where one of the animal genes encoding a neutralization protein was replaced with the human rotavirus gene. These vaccines included Rotashield™ a quadrivalent reassortant vaccine that used a monkey rotavirus as the reassortant virus and Rotateq™ a pentavalent reassortant vaccine that uses a bovine rotavirus for reassortment. Rotashield™ was licensed briefly in the United States before an association with intussusception was found.[7,8] Rotateq™ has recently been licensed after a large safety and efficacy trial revealed no association with intussusception.[9,10] A less common approach to the development of rotavirus vaccines was the use of attenuated human strains.

The RIX4414 vaccine evaluated in the article by Araujo et al.[11] was developed from a rotavirus strain isolated in Cincinnati, OH (strain 89-12) from an ill rotavirus-infected child in 1989. It was found that natural infection with 89-12-like rotavirus induced neutralizing antibodies to the four major rotavirus serotypes and provided protection from subsequent rotavirus infections even if the initial infection was asymptomatic.[12] Recognizing that there might be advantages in developing a vaccine from a human rather than an animal rotavirus strain, multiple cell culture passages were used to attenuate the 89-12 isolate.

This 89-12 isolate was passaged 26 times in primary cells and then seven times in a serially-passaged cell line.[13] The vaccine was then evaluated in phase 1 trials in adults, followed by children with previous rotavirus infections, followed by infants.[13] The vaccine appeared to be safe and efficacy trials followed. In the initial randomized placebo-controlled double blind trial, two oral doses of 89-12 ($1x10^5$ PFU/mL) were safe although a mild fever of short duration was detected in 19% of vaccinees.[14] Efficacy was 89% against any rotavirus disease and 100% against very severe diseases and infections requiring medical attention. Follow-up of the children continued for a second year and revealed an efficacy of 76% against any rotavirus disease and continued to be 100% for very severe disease and infection requiring medical intervention.[15]

Further development of the vaccine utilized limiting dilution cloning of 89-12 in Vero cells and continued passage of the strain. This resulted in a vaccine named RIX4414. This vaccine was then formulated as a lyophilized preparation to be given in a two-dose schedule after reconstitution with a liquid calcium carbonate buffer produced by GlaxoSmithKline. Several safety and efficacy trials revealed the vaccine was safe and did not produce the low grade fever seen with 89-12.[16]

In the excellent report by Araujo et al., the results obtained in Belem, Brazil, in a dose ranging efficacy study of RIX4414 (now licensed as Rotarix™, GlaxoSmithKline) conducted in Brazil, Mexico and Venezuela are reported. These results confirm and enhance previous reports of this vaccine. Most importantly, the detailed follow-up of these infants shows that the vaccine is safe and did not cause excess fever, vomiting, or diarrhea compared to the group given routine vaccinations without the rotavirus vaccine. Further evidence of the safety of this vaccine is detailed in the large study reported in the New England Journal of Medicine.[17] This large study of over 60,000 infants showed that this vaccine was not associated with intussusception. Thus, unlike the previous rotavirus vaccine licensed in the United States, Rotashield™ (Wyeth Ayerst) RIX4414 was not associated with intussusception immediately following vaccine or at any time.

The immunogenicity of the vaccine was also evaluated in the current report and shows that the vaccine induced IgA antibody in about 40% of recipients after one dose and 70% after two doses. This is somewhat lower than previously seen, but as the authors point out, compatible with the reduced immunogenicity sometimes seen with oral vaccines in other developing settings. Most importantly, the vaccine did not interfere with other routine childhood vaccines providing assurance that it can be given as part of the routine immunization schedule.

The article by Araujo et al. also describes the efficacy provided by this vaccine against all cases of rotavirus gastroenteritis, but especially against severe disease and that leading to hospitalization. The highest two doses of the vaccine tested provided 53.9 to 81.5% protection against severe disease and 81.2 to 93.0% protection against hospitalization due to rotavirus gastroenteritis. This is comparable to the efficacy seen in the large trial conducted in Latin America and Finland[17] with a dose similar to the highest dose used in the reported trial.

Perhaps the most important finding reported by Araujo et al. was the efficacy against G9 strains of rotavirus. There has long been a debate about heterotypic rotavirus protection and whether the G1 rotavirus strain contained in the vaccine would protect against other rotaviruses that were not G1. This is now clearly answered in this study and in the larger trial.[17]

The outer shell of the rotavirus is composed of the VP7 (also referred to as the G protein) and VP4 (also referred to as the P protein) proteins. Antibodies to these two proteins, and only these two proteins, are capable of neutralizing the virus *in vitro*. There are at least 14 types of the VP7, with five of them being common in human disease (G1-4 and G9), in

addition to three others (G12, G8 and G5) that have been recently detected. G1 viruses are the most frequently detected. There are at least 25 VP4 types, three of which are seen in humans (P [8], P [4], and P [6]). The most common combinations of VP7 and VP4 are G1P[8], G3P[8], G4P[8], G9P[8] G9P[6], and G2P[4]. True heterologous protection would be against a virus that differs in both of these proteins. Protection by the G1P[8] strain found in Rotarix™ against a G2P[6] infection would be an example of the heterologous protection, while protection of G1P[8] against G3P[8], G4P[8] and G9 P[8] would not be considered truly heterologous because they share the VP4 protein. Thus, the protection against G9 reported here may have come from the antibodies induced by the vaccine VP4 P[8] against other P[8] rotavirus strains, for example, G9P[8].

In summary, the trial reported in this journal supports the safety and efficacy of RIX 4414 (Rotarix™) when given with other routine childhood vaccines. Efforts are continuing to evaluate the vaccine in the least developed areas of the world where the vaccine is most needed.

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