Rotarix: Development of a Live Attenuated Monovalent Human Rotavirus Vaccine

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The public health burden of rotavirus disease, reviewed in the article by Zahn and Marshall (see page 23), provides sufficient justification for the development of a rotavirus vaccine. Despite the withdrawal of one vaccine in the United States, rhesus rotavirus vaccine tetravalent (RRV-TV, RotaShield), other rotavirus vaccines should be available shortly. This article reviews the background, history, and clinical studies of a live attenuated human rotavirus vaccine (LA-HRV, Rotarix) that has already been licensed in several countries.

RATIONALE BEHIND LA-HRV

The rationale behind LA-HRV, and indeed behind all live oral rotavirus vaccines, comes from studies of the protection afforded by natural infection. Both human and animal data show that previous rotavirus infections are highly protective against subsequent rotavirus illness. As early as 1983 — early in rotavirus terms, as the virus was only discovered in 1973 — it was shown that neonatal rotavirus infection provided significant protection against subsequent disease, albeit not against infection per se.1

In perhaps the best study to date of protection following natural infection, it was shown that each rotavirus infection lowers the risk of subsequent infection as well as clinical disease.2 In fact, no child in that study had moderate to severe rotavirus diarrhea after two rotavirus infections, whether those infections were symptomatic or asymptomatic. This was true even though all four major rotavirus serotypes (G1, G2, G3, and G4) circulated during the study period. This observation contributed to the rationale for developing a two-dose vaccine.

A study conducted in Cincinnati in the early 1990s also suggested that a previ-
ous rotavirus infection protected against both symptomatic and asymptomatic reinfections. However, serotype G1 rotavirus predominated in each year of the study, leaving open whether the same protection would have been seen against other serotypes (this is called heterotypic protection). Subsequent studies suggested that this is probable. For example, in a large, multi-center US study, a previous rotavirus infection was 93% effective in preventing symptomatic reinfection, even though the serotype distribution changed from 93% G1 in the first year to 66% G1 in the second year (ie, 34% of the circulating strains were non-G1). In fact, no symptomatic non-G1 serotype reinfection was detected in study participants who had a natural rotavirus infection during the first year.

Still, many questions remain regarding immunity to rotavirus and homologous versus heterologous protection in particular. Because LA-HRV is a monovalent vaccine, this question is especially important. In a study in Bangladesh, children who presented with a clinically significant rotavirus illness generally had substantially lower levels of rotavirus-specific neutralizing antibody than age-matched well children, but only neutralizing antibodies to heterologous and not homologous rotavirus strains correlated to protection in multivariate logistic regression analysis. Several other studies also failed to detect a correlation between serotype-specific serum neutralizing antibody and protection following vaccination.

Interestingly, early vaccine candidates showed inconsistent results, with evidence for homologous but not heterologous protection. In some of these studies, a correlation with rotavirus serum IgA was detected. This indicates that perhaps it is the magnitude of the immune response, rather than specific neutralizing antibody titers, that best correlate with protection, or that factors other than neutralizing antibody, such as local or serum IgA, T-cells, or cytokines can provide protection. Support for protection by each of these immune mechanisms also may be found in mouse and germ-free pig experiments. However, it should be pointed out that second episodes of severe rotavirus disease are more often as-
associated with serotypes that differ from the first infection.2,16

It is clear that intestinal neutralizing antibody can provide protection, but the role of other immune mechanisms is less well accepted. Studies of mice, pigs, and calves have given conflicting results regarding serotype-specific protection and the absolute role of neutralizing antibody.12,13,17,24 For example, it is clear that protection can be provided by vp6, the most abundant rotavirus protein and one that is highly conserved, whether through active immunization with the protein or administration of passive antibody. However, antibodies to vp6 do not neutralize the virus in vitro.

In one study, passively administered IgA but not IgG monoclonal antibody to vp6 provided protection.20 In other murine studies, mucosal immunization with vp6 combined with an adjuvant provided protection.21,22 The mechanism of this protection is not entirely clear but appears to be mediated by CD4-positive T cells.23 There is other evidence that protection can be provided by CD4-positive T cells,24 as well as by CD8-positive T cells25-27 and cytokines.27,28 Thus, it is probable that vaccines that induce high levels of IgA antibody, or cross-reactive T cells, could provide homologous and heterologous protection.22,29

**INITIAL DEVELOPMENT OF LA-HRV**

In 1988, a study evaluating the efficacy of an early bovine-derived vaccine was begun in Cincinnati.30 Serendipitously, the 1988-1989 rotavirus season in Cincinnati was unusual in that only one strain, a G1[P8] serotype, circulated in the community. Although the vaccine proved to be ineffective, the clinical trial provided an opportunity to follow participants prospectively through to the next season and to assess the protection afforded by that single circulating strain of natural rotavirus.3

During the trial, an interesting observation was made. Of the 60 study participants infected during the vaccine trial with the natural strain (20 of these were completely asymptomatic), only four were reinfected (two symptomatic and two asymptomatic) - far fewer infections than among infants with no prior rotavirus experience. It therefore appeared that the strain circulating in Cincinnati that year could provide protection from rotavirus, even if the initial infection was asymptomatic.

Infection with this strain induced more neutralizing antibody to the vp4 (or P) protein than the vp7 (or G) protein, and induced neutralizing antibodies to at least the four major rotavirus serotypes, G1 through G4.31,32 Recognizing that there might be advantages to a vaccine derived from a human rather than an animal rotavirus strain, it was decided to use a single isolate of this strain named 89-12 for further vaccine development.

However, natural strains produce disease and therefore must be attenuated before they can be used as vaccines. The strategy used for 89-12 was to passage it multiple times in tissue culture, the same technique used to produce the Sabin oral polio vaccine. Passage leads to multiple mutations as the virus adapts to growth in tissue culture. Eventually, it was hoped that the accumulation of these mutations would lead to a strain that replicates well in tissue culture but does not produce symptoms in humans.

Thus, 89-12 was passaged 26 times in primary African Green Monkey kidney cells (AGMK), then seven times in a serially passaged AGMK cell line.32 To determine if the passages in cell culture had attenuated the virus, studies were first conducted in adults and then in older children who had had rotavirus infection in the past.33 These trials provided evidence of attenuation based on the lack of signs and symptoms of rotavirus disease, allowing safety and dose-ranging studies to be performed in infants.

In an initial multi-center, randomized, placebo-controlled, double-blind efficacy trial, 213 healthy infants ages 10 to 16 weeks were given 2 oral doses of the vaccine or a placebo following administration of an antacid.34 Antacids have been used with most live rotavirus vaccines to increase the survival of the vaccine virus as it passes through the stomach. The vaccine was found to be safe, although a mild fever of short duration was detected after the first dose in 19% of recipients, compared with 5% of placebo recipients. Rotavirus-specific antibody responses developed in 94% of vaccine recipients, and vaccine virus was detected in 75% of stools obtained on day 7 after immunization.

In the first year following vaccina-
tion, efficacy was 89% against any rotavirus disease, the highest reported for any multi-center trial. Most important, efficacy was 100% against very severe infections and those requiring medical attention. Follow-up of the children for a second year showed that efficacy against very severe disease and illness requiring medical attention remained 100%, and efficacy against any rotavirus disease was 76%.35

**ROTARIX**

Further development of this vaccine was performed by the pharmaceutical company GlaxoSmithKline. The prototype strain was purified by limiting dilution cloning in Vero cells and further passaged in tissue culture. The final product, initially called RIX4414 but now carrying the trade name Rotarix, was formulated as a lyophilized preparation to be given after reconstitution with a liquid calcium carbonate buffer.36

The initial trials of Rotarix established its safety in adults and previously infected toddlers. In a dose escalation study in infants, vaccination was separated from routine immunizations by 2 weeks to allow the most accurate assessment of side effects.37 No increase in any solicited symptom (including fever, diarrhea, or vomiting) was detected at any vaccine dose. Thus, it appeared that the increased passage in tissue culture, the clonal selection process, or both had produced a vaccine strain that was not associated with fever, as was the prototype strain. A dose range effect was seen for the immune response, with seroconversion detected in 73% to 96% of infants after the second dose. Shedding was detected in 38% to 60% of recipients after the first dose, whereas after the second dose, only 0% to 13% of the vaccinees shed the vaccine virus.

Evaluation of Rotarix began in Asia shortly thereafter. At the same time, an intense effort was initiated (mainly centered in Latin America) to evaluate the epidemiology, disease burden, and economic effects of rotavirus disease. In a dose ranging trial conducted in Singapore in 2,464 infants, the vaccine was administered concurrently with routine childhood immunizations.38 Again, no increase in fever, diarrhea, vomiting, or irritability was detected, even at the highest test dose. Similar to the Finnish study, the vaccine was highly immunogenic, with “take” rates (defined as seroconversion or shedding) of nearly 100%. There was no observed interference with the other routine childhood vaccines. More safety and immunogenicity trials have been conducted in South Africa, but results are not yet available.

In a multi-center study conducted in the US, infants ages 5 to 15 weeks received either placebo or $10^{6.2}$ or $10^{6.4}$ focus forming units (ffu) of Rotarix concomitantly with other childhood vaccines.39 The vaccine appeared safe, with no increase in fever, vomiting, diarrhea, or any unsolicited adverse event detected in the vaccine groups (Figure). Vaccine

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**TABLE.**

<table>
<thead>
<tr>
<th>Season</th>
<th>Severity of Disease</th>
<th>Rotarix Gastroenteritis</th>
<th>Placebo Gastroenteritis</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>Any</td>
<td>3%</td>
<td>11%</td>
<td>73%</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>0.4%</td>
<td>4%</td>
<td>90%</td>
</tr>
<tr>
<td>Second</td>
<td>Any</td>
<td>2%</td>
<td>9%</td>
<td>73%</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>0.8%</td>
<td>5%</td>
<td>83%</td>
</tr>
<tr>
<td>Entire follow-up period</td>
<td>Any</td>
<td>5%</td>
<td>19%</td>
<td>72%</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>1%</td>
<td>8%</td>
<td>85%</td>
</tr>
</tbody>
</table>
take was detected in 82% and 88% of the low- and high-dose vaccine recipients, respectively, after 2 doses. Further, the rotavirus vaccine did not interfere with the immune response to the concomitantly administered vaccines.

EFFICACY

The first efficacy study with Rotarix was conducted in Finland over two rotavirus epidemic seasons, 2001 and 2002, with a relatively low dose of vaccine virus (10^4.7 ffu). In this randomized, double-blind, placebo-controlled trial of 405 infants, the vaccine was well tolerated and immunogenic. As seen in the Table (see page 41), in the first season, efficacy against any episode of rotavirus gastroenteritis was 73%, while efficacy against any severe episode was 90%. Over the entire follow-up period, efficacy was 72% against any episode and 85% against severe episodes. No decrease in efficacy was seen in the second year compared with the first. Thirty-five of the 39 cases of rotavirus gastroenteritis were the same serotype (G1) as the vaccine.

In dose-ranging studies conducted in Latin America that have yet to be published in detail, efficacy estimates against severe rotavirus disease reached 86%. Rotavirus-related hospitalizations were reduced from 3% in placebo recipients to 0.6% in vaccine recipients, with a peak efficacy of 93%. Of most importance, vaccine efficacy against non-G1 rotavirus disease, most commonly G9, was similar to the efficacy against G1 serotypes, confirming the hypothesis that the vaccine would provide cross-protection against nonvaccine vp7 serotypes.

INTUSSUSCEPTION

The weak but real association between RotaShield administration and intussusception necessitated that very large safety trials be conducted with new candidate rotavirus vaccines. Rotarix therefore was evaluated in a randomized, placebo-controlled trial of more than 63,000 infants in Latin America. In that trial, 31,373 infants ages 6 to 17 weeks were randomized to receive two doses of vaccine and 31,552 to receive placebo. The mean age at first dose was 8.2 weeks and at second dose was 15.8 weeks.

The primary endpoint of the study was the occurrence of intussusception within 30 days of either dose of vaccine. A secondary evaluation involved cases occurring during the duration of the study. Cases were captured by active hospital surveillance and scheduled visits after each dose. An independent surveillance system was also in place to ensure that all cases were identified, and cases were reviewed by a blinded expert and monitored by an independent data monitoring board.

Seven cases of intussusception (two following the first dose) were identified in the placebo group, compared with six (one following the first dose) in the vaccine group. No clustering in the initial 1 to 2 weeks was identified after either dose. Across the duration of the study, there were 16 cases in the placebo group, compared with 9 in the vaccine group. Thus, there was no evidence that Rotarix was associated with intussusception.

CURRENT STATUS

Rotarix is licensed in Mexico, the Dominican Republic, and Kuwait and is awaiting approval in other Latin American countries, the European Union, and several countries in the Asian-Pacific region. Discussions with the Food and Drug Administration regarding licensure in the US are ongoing.

The evidence indicates that two oral doses of Rotarix are safe and are not associated with significant side effects. Most important, a large safety trial showed no association with intussusception. Efficacy studies have indicated excellent protection, especially against severe disease and hospitalization due to rotavirus, including those caused by non-serotype G1 infections. Trials currently under way will determine if the ef-

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REFERENCES


