Reappraisal of the Peruvian and Brazilian lower titer tetravalent rhesus-human reassortant rotavirus vaccine efficacy trials: analysis by severity of diarrhea

ALEXANDRE C. LINHARES, MD, CLAUDIO F. LANATA, MD, MPH, WILLIAM P. HAUSDORFF, PHD, YVONE B. GABBAY, BS, MS AND ROBERT E. BLACK, MD, MPH

Objectives and methods. With the purpose of better understanding the efficacy of the lower titer [4 × 10^4 plaque-forming units (pfu)] tetravalent rhesus-human reassortant rotavirus vaccine (RRV-TV) against diarrheal episodes of different severities, the Peruvian and Brazilian efficacy data were reanalyzed with a 20-point scoring system. Mild, moderate/severe and very severe rotavirus diarrhea were scored as 0 to 8, 9 to 14 and >14, respectively.

Results. In the Peruvian study one dose of vaccine yielded 64% (P = 0.04) protection against pure cases of rotavirus disease (i.e. those in which no other enteropathogen was found) with clinical scores ranging from 9 to 14. Protective efficacy against very severe rotavirus gastroenteritis could not be assessed because of the small number of cases. In Brazil there was a trend in preventing “all” and “pure” cases of rotavirus diarrhea scored 9 to 14 (44%, P = 0.06, and 45%, P = 0.08, respectively) and the vaccine was 75% (P = 0.02) protective against pure rotavirus diarrhea scored >14. No protection was observed for mild rotavirus diarrhea (scores < 9). These data were compared with those from trials in Venezuela (4 × 10^5 pfu/dose), US (4 × 10^4 pfu/dose and 4 × 10^5 pfu/dose) and Finland (4 × 10^5 pfu/dose). Combining the Peruvian (one dose, pure cases) and Brazilian studies together, the levels of protection against 9- to 14-scored rotavirus diarrhea are comparable with those from the Venezuelan (47%) and American (57, 57 and 65%) efficacy trials. In Brazil the level of protection (75%) against pure, >14-scored rotavirus diarrhea is similar to the efficacy rates yielded in the three US trials (82, 80 and 69%) and the Finnish trial (100%) for episodes of the same severity.

Conclusions. Our reanalysis provides evidence that, at least against moderate/severe rotavirus gastroenteritis, RRV-TV, 4 × 10^4 pfu/dose is potentially as efficacious as RRV-TV, 4 × 10^5 pfu/dose, even in settings with very high rotavirus disease burden. The reanalysis of the Peruvian data suggests that one and three vaccine doses may yield similar efficacy rates. It is also suggested that vaccine efficacy against most severe episodes in Peru and Brazil was not evident because of the trial design used in those studies (i.e. prospective, active home surveillance rather than a catchment trial), resulting in too few cases of severe disease even in the placebo group. To confirm these findings, future trials with this vaccine are necessary in developing countries with high diarrhea morbidity rates. These trials should use catchment designs and focus on the evaluation of the efficacy of one or three doses of RRV-TV against moderate to severe/very severe rotavirus diarrhea.

INTRODUCTION

Rotavirus has been recognized worldwide as the single most important cause of severe watery diarrhea among infants and young children, infecting virtually every child by the age of 4 years.1 The vast public health burden associated with rotavirus disease can be quantified by estimates reporting the occurrence of >125 million cases of infantile gastroenteritis and nearly 1 million deaths annually, mostly in developing areas.2,3 For this reason development of an effective rotavirus vaccine targeted for use in early childhood has been given high public health priority. It would be optimal to prevent infection and all illness, but if that
is not possible there would still be great value in preventing severe disease.4

Several strategies have been attempted to develop a vaccine, ranging from the “Jennerian approach,” using rotavirus strains of animal origin, to the application of molecular biologic techniques.5, 6 The most extensively evaluated candidate vaccine is a tetravalent preparation that combines the rhesus-human reassortants specific for G serotypes 1, 2 and 4 with the rhesus rotavirus itself (RRV) as a component having homology with the human G type 3,7, 8

To date seven efficacy trials with the lower9-11 and higher12-15 tetravalent rhesus-human reassortant rotavirus vaccine (RRV-TV) titer formulations have been completed in 5 countries, involving >8000 infants ages 1 to 6 months. In general protective efficacy rates were reported to be higher in the US, Finland and Venezuela than they were in Peru and Brazil, and the vaccine appeared to selectively protect against the most severe rotavirus disease.16, 17

In all those trials the use of the severity scoring system allowed cross-trial comparisons and made it apparent that there is a gradient of efficacy with the RRV-TV, with the vaccine most effective in preventing the most severe disease, least effective in preventing mild disease and with intermediate effectiveness against disease of moderate severity. However, in the original reports of Peru and Brazil studies, trial results were generally not presented using the scoring system and, therefore, only limited conclusions could be drawn regarding efficacy according to severity. In those studies three doses of a lower titer of the same vaccine in previous (longitudinal, community-based) trials involving children from poor urban areas of Lima, Peru, and Belém, Brazil, 9, 10 provided only partial protection against any rotavirus diarrhea (24% and 35%, respectively). When analyzed against moderate/severe episodes of rotavirus diarrhea, the relative efficacies of three doses were 30 and 46% for the Peruvian and Brazilian trials, respectively.

In the US, Finland and Venezuela studies, clinical severity of rotavirus diarrhea was primarily assessed by a 20-point scoring system modified from that of Flores et al.14 For the Peru and Brazil trials, we previously assessed the protective efficacy of the RRV-TV vaccine against more severe rotavirus disease. Rates were compared among the vaccine and placebo groups by using the Mantel-Haenszel chi square test of association or Fisher’s exact test, as appropriate. Significance was defined as P < 0.05.

MATERIALS AND METHODS

Subjects, vaccine and study design. The methodology of the efficacy studies conducted in Lima, Peru, and Belém, Brazil, has largely been described elsewhere.9, 10 Briefly the former trial involved 700 infants who were given, at the ages of 2, 3 and 4 months, either 3 doses of RRV-TV [4 x 104 plaque-forming units (pfu)], an initial dose of vaccine and then placebo at 3 and 4 months, or 3 doses of placebo. In the Brazilian efficacy trial either 3 doses of the RRV-TV (4 x 104 pfu) vaccine or the placebo were administered to 540 infants 1, 3 and 5 months old. In both studies a prospective, active surveillance for diarrheal episodes was carried out through twice-a-week home visits made by trained field workers. Mothers were provided with instructions regarding the use of oral rehydrating salts, and whenever signs of dehydration were identified by field workers children were visited by a physician or referred to either a health clinic or a hospital. In the Belém study, once a child showed signs of gastroenteritis daily visits were made until the episode ended. In this reanalysis both Peruvian and Brazilian data were compared with published results from other efficacy studies in Venezuela, the US and Finland. The only unpublished data were those from one of the US trials15 with respect to mild and moderate/severe diarrheal episodes (information kindly provided by Dr. M. Santosham, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD).

Scores for clinical severity of diarrhea. For the Peruvian, Brazilian and US efficacy studies, the severity of rotavirus gastroenteritis was graded by a 20-point scoring system (Table 1, Score A) modified from that of Flores et al.14 A similar 20-point scoring system (Table 1, Score B), as proposed by Ruuska and Vesikari,19 was used in the vaccine trials conducted in Venezuela13 and Finland.12 Cases of rotavirus gastroenteritis with clinical scores of 0 to 8, 9 to 14 and >14 were defined as mild, moderate/severe and very severe, respectively. Vaccine efficacy was determined for any rotavirus-positive diarrheal episode and for episodes of pure rotavirus infection, i.e. those in which no other enteropathogen was identified.

Statistical analysis. Analyses of the data were done originally using the SPSS software (SPSS, Chicago, IL). The vaccine efficacy and 95% confidence intervals were determined as previously recommended.20 Rates were compared among the vaccine and placebo groups by using the Mantel-Haenszel chi square test of association or Fisher’s exact test, as appropriate. Significance was defined as P < 0.05.
RESULTS
The protective efficacy of RRV-TV (4 × 10⁴ pfu/dose) vaccine in Peru and Brazil against all episodes and pure episodes of rotavirus diarrhea, according to clinical severity, is summarized in Table 2. A comparison of these results is also made with the protection rates achieved in five other trials from three countries. In the Peruvian study, one vaccine dose and three vaccine doses provided 64% (\( P = 0.04; 95\% \) confidence interval (CI), 1 to 87) and 19% (\( P = 0.59; 95\% \) CI, -75 to 63), respectively, against moderate/severe rotavirus diarrhea. There were too few cases of either all or pure very severe rotavirus diarrhea to evaluate efficacy in Peru.

In Brazil moderate protection was achieved against all and pure cases of rotavirus gastroenteritis with clinical scores ranging from 9 to 14, after three doses of the lower titer RRV-TV: 44% (\( P = 0.06; 95\% \) CI, 0 to 70) and 45% (\( P = 0.08; 95\% \) CI, -9 to 73), respectively. In this study the RRV-TV provided 75% (\( P = 0.02; 95\% \) CI, 12 to 93) protection against the most severe pure episodes of rotavirus gastroenteritis (severity score, >14). Taking the Peruvian (one dose, pure cases) and Brazilian figures together, the protection/trend for protection against 9- to 14-scored diarrhea is comparable with the efficacy rates yielded in Venezuela (47%) and in (three) US trials (57%, 4 × 10⁴ pfu/dose; 57%, 4 × 10⁵ pfu/dose; and 65%, 4 × 10⁵ pfu/dose), but there was a trend for lower protection than that of the Finnish study (78%). With respect to the pure, most severe rotavirus gastroenteritis (episodes scored >14), the protective efficacy achieved in Brazil (75%) is similar to the rates of protection in the US trials (82%, 4 × 10⁴ pfu/dose; 80%, 4 × 10⁵ pfu/dose; and 69%, 4 × 10⁵ pfu/dose) and tended to be lower than those for the Venezuelan (90%, 4 × 10⁴ pfu/dose) and Finnish (100%, 4 × 10⁴ pfu/dose) trials. No vaccine protection was observed against mild rotavirus diarrhea (clinical scores <9).

DISCUSSION
The reevaluation of the efficacy data from the Peruvian and Brazilian studies, based on a wider spectrum of clinical severity, suggests that RRV-TV may protect against severe rotaviral disease even in the highly endemic environments. Only pure episodes of rotavirus diarrhea were considered across the studies under comparison, because mixed infections are much more common in developing countries and this might confuse intertrial comparisons. Although using a 10-fold lower titer of the same vaccine, the study in Brazil showed rates of protection against pure rotavirus diarrhea that scored 9 to 14, which were comparable to those of trials conducted in Venezuela and US. However, there is a wider confidence interval of results from the former study, if compared with the latter ones. The reanalyzed point estimate for efficacy after one dose in the Peruvian trial also gave a comparable result, although with a wide confidence interval because of the small number of cases. The 75% protective efficacy of RRV-TV in Brazil against very severe pure rotavirus gastroenteritis approaches the efficacy rates against diarrheal episodes of similar clinical severity achieved by a 10-fold higher vaccine titer in Venezuela and US. In general our data support the view that rotavirus vaccines induce a greater protection against severe than mild diseases, an observation that has been made since
TABLE 2. Protective efficacy of RRV-TV (4 × 10^6 pfu/dose) against rotavirus gastroenteritis, according to clinical severity scores,* in Lima, Peru, and Belém, Brazil, and comparison with efficacy data from other trials in developing and developed countries

<table>
<thead>
<tr>
<th>RV Diarrhea Episodes by County</th>
<th>No. of Doses</th>
<th>No. of Infants</th>
<th>No. Episodes with Clinical Score</th>
<th>% Protection against Clinical Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plac</td>
<td>V acc</td>
<td>8–10</td>
</tr>
<tr>
<td>All cases</td>
<td></td>
<td>Plac</td>
<td>V acc</td>
<td>8–10</td>
</tr>
<tr>
<td>Peru†</td>
<td>1 (4 × 10^6)</td>
<td>38.6†</td>
<td>38.2†</td>
<td>22</td>
</tr>
<tr>
<td>Peru‡</td>
<td>2 (4 × 10^6)</td>
<td>38.6‡</td>
<td>37.6‡</td>
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</tr>
<tr>
<td>Brasil§</td>
<td>3 (4 × 10^6)</td>
<td>36.3§</td>
<td>36.1§</td>
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<td>Pure cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peru†</td>
<td>1 (4 × 10^6)</td>
<td>38.6†</td>
<td>38.2†</td>
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<td>Peru‡</td>
<td>3 (4 × 10^6)</td>
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<tr>
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<td>36.3§</td>
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<td>All cases</td>
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<tr>
<td>Venezuela†</td>
<td>3 (4 × 10^6)</td>
<td>1095</td>
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<td>Pure cases</td>
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<tr>
<td>Venezuela†</td>
<td>3 (4 × 10^6)</td>
<td>1095</td>
<td>1112</td>
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<tr>
<td>Finland12</td>
<td>3 (4 × 10^6)</td>
<td>1207</td>
<td>1191</td>
<td>47</td>
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</table>

* A twenty-point scoring system modified from that of Flores et al.13 was used for studies in Peru, Brazil and the US; and clinical severity data from Venezuela and Finland are presented as been on the scoring system of Roursa and Vesikari.14
† Number in parentheses, vaccine titer
‡ Number of child years.
§ Numbers in parentheses, 95% CI.
¶ P < 0.05.
|| Efficacy rates for the first year of surveillance; no protective efficacy during Year 2.
** Based on the Poisson distribution.

Although two scoring systems were used in studies under comparison,16, 19 the levels of protection did not change significantly when efficacy data in the Belém study, for example, were reassessed by the same score used in the Venezuelan and Finnish trials. The use of 20-point scoring systems provides a more accurate assessment of protective efficacy than individual endpoints, thus minimizing the possible influence of local differences in treatment or referral patterns. In addition systems comprising multiple indicators of clinical severity, such as those used in the present reanalysis, provide a more comprehensive assessment of disease severity, rather than relying on any single one criterion.

Because the present reanalysis has led to a change in the main conclusions drawn from the originally published studies,9, 10 caution should be exercised when interpreting its results. Although this reanalysis has clearly demonstrated the benefits of reevaluating RRV-TV efficacy, according to clinical severity subgroups, its retrospective nature strongly indicates the need to confirm such findings through the conduct of additional efficacy trials in developing countries.

A possible explanation for the overall lesser efficacy rates achieved in Peru and Brazil is the low incidence of very severe disease, because of the frequent interventions in these longitudinal, active surveillance studies. In this respect it should be pointed out the services provided by the trained field workers, who not only gave regular advice on the management of diarrhea at the home level but also made oral rehydration solution readily available during their frequent home visits. For this reason it has been difficult to draw firm conclusions concerning the efficacy of RRV-TV against >14-scored rotavirus disease in both Peruvian and Brazilian studies. In contrast with these two studies the catchment trial conducted in Venezuela, for example, using the 10-fold higher RRV-TV titer, tended to focus on the severe/very severe disease, because cases of diarrhea were detected, through passive surveillance, at the hospital.13 Although the rates of moderate/severe dehydration were low (<5%) (even in the placebo group) during the trials in Peru and Brazil,9, 10 this condition has been diagnosed in up to 20% of cases of rotavirus diarrhea in other community-based studies carried out in a similar setting in Brazil, for example, where intervention was not frequent (fortnightly home visits).21
The present reanalysis of the Peruvian data suggests that one vaccine dose may be at least as effective against rotavirus diarrhea as the widely used three dose regimen. In a population heavily exposed to rotavirus infection, as observed in Lima and Belém, it is likely that protection conferred by one vaccine dose given early in life may be enhanced by subsequent natural (mostly asymptomatic) infections, as based on findings from previous studies in Mexico. Previous one vaccine-dose immunogenicity studies with the lower titer RRV-TV in Venezuela yielded enzyme-linked immunosorbent assay IgA seroconversion rates ranging from 63.23 to 74%. which were similar to those of three dose regimen trials, with the same vaccine, conducted elsewhere. In addition previous efficacy trials in developing countries with vaccines other than RRV-TV have shown that one dose may be sufficient to achieve rates of protection as high as 85 to 90% against severe episodes of rotavirus gastroenteritis. If possible future trials prove that one dose, rather than three, is sufficient to confer a good protection in countries like Brazil and Peru, this would be of importance when assessing cost effectiveness of immunization programs in the poorer regions of the world. This is because it is likely that the price for this (and other new) vaccine(s) in developing countries will be significantly more than for the older Expanded Program on Immunization vaccines, even after probable price tiering by manufacturers.

An issue that also deserves particular consideration when analyzing the Peruvian data is the apparent lack of efficacy with three doses of RRV-TV, as compared with the Brazilian trial. Although this may have occurred by chance it would be worth considering two other possible explanations for such a difference: (1) the active surveillance efforts in Peru may have eliminated almost all severe diseases in the placebo group; and (2) the severity distribution of the 9- to 14-scored diarrheal episodes in both studies are different; indeed in the placebo group in the Brazil trial one-third of cases in the 9 to 14 interval scored 13 or 14, but in the Peruvian placebo group less than one-seventh scored that (data not shown in tables).

Because there is a particular interest in assessing the performance of RRV-TV vaccine against life-threatening diarrheal episodes, the efficacy trial design seems to be of considerable importance in the detection of those most severe (clinical scores >14) cases of rotavirus gastroenteritis. In this respect and in view of recent findings, it could be postulated that a greater vaccine efficacy against these more severe diarrheal episodes should offer an additional, indirect benefit in reducing both the secondary spread of disease and the post-gastroenteritis syndrome.

Our reanalysis indicates that lower titer RRV-TV was potentially efficacious against severe rotavirus disease in Peru and against severe and very severe rotavirus diarrhea in Brazil. It is also suggested that design for future vaccine studies should essentially include passive surveillance at the hospital (catchment trials), with the use of one or three doses of the RRV-TV. These studies are urgently needed to define the role of the RRV-TV vaccine in developing countries with high rates of diarrheal diseases and malnutrition. If these studies confirm our findings (i.e. that the RRV-TV vaccine, even one dose of it, confers a significant protective efficacy against moderate to severe/very severe rotavirus diarrhea), the implementation of this vaccine in developing countries will be warranted. This would include those poorer areas of the world with high incidence of diarrhea, elevated levels of baseline rotavirus antibody and malnutrition.

ACKNOWLEDGMENTS

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Commentary: Reanalysis of the results of two rotavirus vaccine trials: an appraisal of the reappraisal

ROGER I. GLASS, MD

In this issue of the Journal two experienced epidemiologists who each conducted field trials with the tetravalent rhesus rotavirus vaccine have reanalyzed their results and arrived at more positive assessments of vaccine efficacy than either of them concluded in their first publications.1, 2 This idea of reassessing results and conclusions is most unusual for those involved in clinical studies. Indeed, the design issues surrounding randomized clinical trials are full of methods to check, randomize, blind and control to avoid the introduction of bias at every stage in the study from recruitment to final analysis. Moreover, the peer review process for protocol approval and publication is aimed at ensuring that results from important trials can be interpreted in the light of past trials and experience and with the avoidance of bias. Hence the efforts to “reappraise” the results of two independent trials after publication is noteworthy and provides important lessons for others engaged in similar clinical studies.

In their original publications Drs. Linhares and Lanata and their colleagues each conducted independent trials of the new tetravalent rhesus rotavirus vaccine administered in one-tenth the dose that is included in the currently licensed product. The protection reported was mediocre at best. In Brazil the efficacy was 35% against any rotavirus diarrhea, 57% against diarrhea in the first year of follow-up and 63% against severe diarrhea in which rotavirus was the only pathogen and the clinical score identified the most severe cases. In Peru a comparison of one vs. three