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Comparison of virus shedding after lived attenuated and pentavalent reassortant rotavirus vaccine

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ABSTRACT

Transmission of rotavirus vaccine or vaccine-reassortant strains to unvaccinated contacts has been reported. Therefore, it is essential to evaluate and characterize the nature of vaccine–virus shedding among rotavirus vaccine recipients. Two groups of healthy infants who received a complete course of RotaTeq (RV5) or Rotarix (RV2) were enrolled (between March 2010 and June 2011) to compare fecal shedding for one month after each vaccine dose. Shedding was assessed using both enzyme immunoassay (EIA) and real-time reverse transcription-polymerase chain reaction (RT-PCR). Eighty-seven infants (34 girls and 53 boys) were enrolled in the study. After the first vaccine dose, the peak time of virus shedding occurred between day 4 and day 7, with positive detection rates of 80–90% by real-time RT-PCR and 20–30% by EIA. In both groups, vaccine shedding occurred as early as one day and as late as 25–28 days. Mixed effects logistic regression analysis of real-time RT-PCR data showed no significant differences between two groups when shedding rates were compared after the first vaccine dose (odds ratio [OR] 1.26; $P = 0.71$) or after the second vaccine dose (odds ratio [OR] 1.26; $P = 0.99$). However, infants receiving RV2 shed significantly higher viral loads than those receiving RV5 when compared after the first vaccine dose ($P = 0.001$) and after the second dose ($P = 0.039$). In terms of shedding rates detected by real-time RT-PCR, vaccine uptake of RV5 or RV2 among infants in Taiwan was comparable. Clinical significance of higher shedding viral loads in RV2 should be further observed.

1. Introduction

The implementation of rotavirus vaccination has reduced the childhood burden of rotavirus gastroenteritis, a most common cause of severe acute gastroenteritis [1–3]. Currently, two live oral rotavirus vaccines are available against rotavirus gastroenteritis in many countries worldwide. One (RV5; marketed as RotaTeq, Merck, Whitehouse Station, NJ) is a pentavalent (G1, G2, G3, G4 and P [8]) human-bovine (WC3) reassortant vaccine that is administered as a three-dose series. The other (RV2; marketed as Rotarix, GlaxoSmithKline Biologicals, Rixensart, Belgium) is a human-attenuated monovalent vaccine derived from the virulent wild-type strain G1P [8], and is administered as a two-dose series. Post-licensure evaluation has demonstrated that each vaccine is highly effective in preventing rotavirus gastroenteritis-associated hospitalization [4,5]. In some studies, RV2 also provided protection against gastroenteritis caused by heterotypic strains such as G9P [8], and G2P [4,6–8].

Rotaviruses are enteric pathogens that infect and destroy mucosal cells in the small intestine; therefore, rotavirus vaccines are usually designed to be oral live vaccines to best induce a protective immunity that mimics that induced by natural infection. Following vaccination, rotavirus vaccine strains are expected to replicate in the gastrointestinal tract and be shed in stool. Horizontal transmission of vaccine strains to unvaccinated contacts through fecal shedding harbors the potential for herd immunity and provides a substantial benefit, but also risks infection resulting in symptomatic vaccine-derived disease, especially in immunocompromised contacts. In fact, transmission of these two rotavirus vaccines or vaccine-reassortant strains to unvaccinated contacts has been detected [9–13], even in the absence of symptoms. The clinical relevance of vaccine or vaccine-reassortant strains in

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pediatric rotavirus infection needs further study. Therefore, it is essential to evaluate and characterize vaccine–virus shedding following rotavirus vaccination.

The present study was designed to compare fecal shedding, for one month after rotavirus vaccination, between two groups of healthy infants who receiving a complete course of RV5 or RV2. We detected virus shedding by using both enzyme immunoassay (EIA) and real-time reverse transcription-polymerase chain reaction (RT-PCR) to provide a comprehensive understanding of fecal shedding after rotavirus vaccine administration.

2. Materials and methods

2.1. Participants and study design

Participants were infants who had received either type of rotavirus vaccine (RV5 or RV2). The infants were patients at a well-baby clinic in Chang Gung Memorial Hospital or at a local women and children's hospital. During patient visits, pediatricians would explain the characteristics, expense, and dosage of RV5 and RV2 to their primary caretakers. The primary caretakers made the final decision and pay for the vaccine. Study personnel obtained consent for participation from the primary caretakers outside the clinics. Participants were invited to enroll in the study between March 2010 and June 2011. During the study period, infants also received other routine pediatric immunizations in accordance with local recommendations (Infants receive inactivated poliovirus vaccine in Taiwan). The primary caretaker was given stool collection bottles and instructed to collect serial stool specimens every day for 28 days following each vaccine dose. Samples were submitted for detection of rotavirus antigen or RNA until negative results were obtained for two sequential samples. i.e. Participants didn't have to submit stool samples if their stool EIA tests were negative for two sequential samples. The study was approved by the Institutional Review Board of the Chang Gung Memorial Hospital.

2.2. Laboratory testing

Stool specimens were screened for rotavirus VP6 antigen using an enzyme immunoassay (EIA) kit (RIDASCREEN® Rotavirus, R-biopharm AG, Germany), which was performed at Chang Gung Memorial Hospital. Additionally, specimens were shipped to the Rotavirus Reference Laboratory at Centers for Disease Control, Taiwan for genotyping and viral load measurement every week. Stool specimens were stored at −80 °C.

2.2.1. RNA extraction and real-time reverse transcription-polymerase chain reaction (RT-PCR)

Viral RNA was extracted from 10% (w/v) purified fecal supernatants using the MagNA Pure LC DNA isolation kit III (Roche Diagnostics GmbH, Germany) according to the manufacturer's instructions. The extracted RNA was used as the template for real-time RT-PCR. Single-step real-time RT-PCR was performed using the NSP3F/NSP3R primer set as previously described [14].

2.2.2. Viral load measurements

The real-time RT-PCR amplicon was cloned into the pCR2.1-TOPO vector (Invitrogen, USA) as a standard control. Specimen viral load was quantified in duplicate per run and compared to 10-fold serial dilutions of the plasmid standard that were expected to contain from 10^2 to 10^8 copies each. In this assay, the detection limit for rotavirus was equivalent to 10^2 cDNA copies/reaction.

2.3. Statistical analysis

There were several missing data due to unavailable stool samples in the database, therefore we used mixed effects logistic regression to evaluate the difference in shedding rate, as detected by EIA or real-time RT-PCR, between patients vaccinated with RV5 or RV2. Mixed effect model analysis can provide quite robust estimates for repeated measures data, even when data are not completely missing at random [15]. A random intercept approach was used to account for within-subject correlation. We estimated the marginal mean viral shedding loads during the whole course of data collection using the linear mixed model. The dependent variable was the log-transformed viral shedding loads and the independent variables included a categorical factor, which indicated five classifications (RV2, dose 1; RV2, dose2; RV5, dose 1; RV5, dose2; RV5, dose 3) and a time variable, which reflected the sampling days. An auto-regressive correlation structure (AR1) was used to account for temporal correlation among repeated measures. The interaction between group and time was not included in the final model due to the non-significance. The differences in mean viral shedding loads between the targeted groups were linear contrasts estimated from the linear mixed model analysis. A p value less than 0.05 was considered statistically significant. Statistical analysis was conducted using Stata/MP 12.1 for Windows (StataCorp LP, College Station, TX).

3. Results

Eighty-seven infants (34 girls and 53 boys) were enrolled in the study. All infants received a complete course of RV5 or RV2, but the numbers of participants in each dose of vaccine varied. Forty-five participants received RV5. The numbers of participants in dose 1, 2 and 3 of RV5 was 19, 45 and 45, respectively. 42 participants received RV2. The numbers of participants in dose 1, and 2 of RV2 was 40 and 42, respectively. Table 1 shows baseline characteristics of the study participants. At the time of administration of the first dose, the demographic profiles of the two groups did not differ significantly with respect to age, sex, height, or weight (Table 1). To simplify the data analysis, we calculated the shedding rate and viral loads for intervals corresponding to days 0–3, 4–5, 6–7, 8–10, 11–14, 15–21, and 22–28 after of each vaccine dose administration. During day 0–14, the sampling rates of both groups after dose 1 were around 70–90% (Table 2); the sampling rates of both groups after dose 2 were around 60–90%. During day 15–28, the stool sampling rate decreased to 10–30% partially due to our study design which designed that participants did not have to submit stool samples if their stool EIA tests were negative for consecutively twice.

Table 1
Baseline demographic characteristics.

	RV5 Group (n = 45)	RV2 Group (n = 42)	P value
Gender, n (%)			0.49
Male	29 (64.4)	24 (57.1)	
Female	16 (35.6)	18 (42.9)	
Age at entry, wk			0.9
Mean ± SD	11.9 ± 4.5	11 ± 3.4	
Median	10	9	
Range	6–26	6–19	
Body weight at entry, kg			0.7
Mean ± SD	5.96 ± 0.7	6.05 ± 1.1	
Median	5.9	5.9	
Range	4.6–8.0	4.3–8.0	
Body height at entry, cm			0.9
Mean ± SD	59 ± 2.9	59.1 ± 3.5	
Median	59	58	
Range	53.7–67.5	52–66	