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Molecular characterisation of rotavirus strains detected during a clinical trial of the human neonatal rotavirus vaccine (RV3-BB) in Indonesia

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ABSTRACT

Background: The RV3-BB human neonatal rotavirus vaccine aims to provide protection from severe rotavirus disease from birth. The aim of the current study was to characterise the rotavirus strains causing gastroenteritis during the Indonesian Phase IIb efficacy trial.
Methods: A randomized, double-blind placebo-controlled trial involving 1649 participants was conducted from January 2013 to July 2016 in Central Java and Yogyakarta, Indonesia. Participants received three doses of oral RV3-BB vaccine with the first dose given at 0–5 days after birth (neonatal schedule), or the first dose given at ~8 weeks after birth (infant schedule), or placebo (placebo schedule). Stool samples from episodes of gastroenteritis were tested for rotavirus using EIA testing, positive samples were genotyped by RT-PCR. Full genome sequencing was performed on two representative rotavirus strains.
Results: There were 1110 episodes of acute gastroenteritis of any severity, 105 episodes were confirmed as rotavirus gastroenteritis by EIA testing. The most common genotype identified was G3P[8] (90/105), the majority (52/56) of severe (Vesikari score ≥11) rotavirus gastroenteritis episodes were due to the G3P[8] strain. Full genome analysis of two representative G3P[8] samples demonstrated the strain was an inter-genogroup reassortant, containing an equine-like G3 VP7, P[8] VP4 and a genogroup 2 backbone I2-R2-C2-M2-A2-N2-T2-E2-H2. The complete genome of the Indonesian equine-like G3P[8] strain demonstrated highest genetic identity to G3P[8] strains circulating in Hungary and Spain.
Conclusions: The dominant circulating strain during the Indonesian Phase IIb efficacy trial of the RV3-BB vaccine was an equine-like G3P[8] strain. The equine-like G3P[8] strain is an emerging cause of severe gastroenteritis in Indonesia and in other regions.

1. Introduction

Rotavirus is the most common cause of severe gastroenteritis in children under five years of age [1]. The currently available rotavirus vaccines have been introduced into the national immunisation programs of 92 countries globally and reduced hospital admissions and child mortality from gastroenteritis [2–5]. Despite this success, several barriers to global vaccine implementation exist, including cost and sub-optimal efficacy in low-income countries [6]. The human neonatal rotavirus vaccine, RV3-BB, is in clinical development with a birth dose vaccination schedule and is proposed to address some of these barriers. The RV3-BB vaccine is based on a naturally attenuated asymptomatic neonatal G3P[6] rotavirus strain, first identified in Melbourne obstetric hospitals in the 1970s [7]. A randomized, placebo-controlled trial to evaluate the efficacy of an oral human-strain neonatal rotavirus vaccine (RV3-BB) was recently completed in central Java and Yogyakarta, Indonesia [8]. Vaccine efficacy against severe rotavirus gastroenteritis from 2 weeks after dose 3 and to 18 months of age was 63% in the combined vaccine group (95% confidence interval [CI] 34, 80; $p < 0.001$), 75% in the neonatal vaccine group (95% CI 44, 91; $p < 0.001$) and 51% in the infant vaccine group (95% CI 7, 76; $p = 0.03$). The vaccine was also found to be immunogenic and

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well-tolerated when administered in either the neonatal or infant schedules.

The rotavirus strains which circulate in the human population demonstrate significant genetic diversity. The rotavirus genome is comprised of 11 segments of double stranded RNA, encoding six structural (VP1-4, VP6, VP7) and six non-structural proteins (NSP1-5/6) [9]. The segmented genome facilitates reassortment between strains, allowing both intra- and inter-genogroup reassortment. Continued genetic variation by sequential point mutations and zoonotic transmission of novel animal strains also increases the genetic diversity within circulating rotavirus strains causing human infection. A genotype classification system based on capsid genes VP7 and VP4 is used in molecular epidemiology of rotavirus strains denoting the G-type (glycoprotein) and P-type (protease sensitive) respectively [9]. Whole genome classification is also used, the nomenclature Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx represents the genotypes of VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5/6 respectively [10]. Currently there are 35 G, 50 P, 26 I, 21 R, 19 C, 19 M, 30 A, 21 N, 21 T, 27 E and 21 H types [11]. There are two major genotype constellations of human rotaviruses, termed genogroup 1 (G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1), genogroup 2 (G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2) [12].

A more dynamic and diverse rotavirus strain population has been observed in the vaccine era [13,14]. For rotavirus vaccines to be effective they must provide protection against multiple circulating genotypes. Strain diversity may also be a factor in vaccine effectiveness in low- and middle-income countries, which can have a higher diversity of strains and distinct dominant genotypes compared to high-income settings [15]. Therefore, understanding the genetic diversity of the rotavirus strains causing gastroenteritis during the RV3-BB vaccine trial will provide valuable insights for future implementation of this vaccine. During the phase IIb efficacy trial, study participants were followed for episodes of gastroenteritis from birth to 18 months of age. In the present study, we sought to characterise the genetic diversity of strains causing acute gastroenteritis during the trial of the RV3-BB in Indonesia.

2. Method and materials

2.1. Study design and participants

The study design and recruitment for the Phase IIb efficacy, safety and immunogenicity trial of the RV3-BB vaccine has been previously described [8]. Briefly, a randomized, double-blind placebo-controlled trial involving 1649 participants was conducted from January 2013 to July 2016 in primary health centres and hospitals in Klaten, Central Java, and Sleman, Yogyakarta, Indonesia. Eligible infants (healthy, full term babies 0–5 days of age, birth weight of 2.5–4.0 kg) were randomized into one of three groups (neonatal vaccine group, infant vaccine group, or placebo group) in a 1:1:1 ratio according to a computer-generated code (block size = 6) which was stratified by province. The trial protocol was approved by the ethics committees of Universitas Gadjah Mada, Royal Children's Hospital Melbourne and National Agency of Drug and Food Control, Republic of Indonesia.

During the recruitment process, 2405 pregnant women gave antenatal preliminary consent, 1649 were randomized, 549 to neonatal vaccine schedule, 550 to infant vaccine schedule, 550 to placebo schedule. The analysis of vaccine efficacy was performed on per protocol (n = 1513) and intention to treat (ITT) (n = 1649) populations followed for severe episodes of rotavirus gastroenteritis occurring from two weeks post investigational product (IP) dose 4 to 18 months of age [8]. To characterise all rotavirus positive cases the current genotype analysis was performed on the ITT population, and included episodes of rotavirus gastroenteritis of any severity that occurred from administration of the birth dose until 18 months of age.

The investigational product (IP) consisted of RV3-BB vaccine ($8.3–8.7 \times 10^6$ FCFU/ml) or Placebo (cell culture medium, DMEM). RV3-BB clinical trial lots were prepared at Meridian Life Sciences (Memphis, USA) to a titre of $8.3–8.7 \times 10^6$ FFU/mL in serum free media supplemented with 10% sucrose. Placebo contained the same media with 10% sucrose and was visually indistinguishable. Vials were stored at −70 °C until thawed within 6 h prior to administration.

2.2. Sample collection and processing

The participant's parent(s)/guardian(s) were asked to collect at least two faecal samples per diarrhoea episode, from separate stools. Samples were obtained using faecal spatulas to scrape at least two scoops of faeces from infants' skin or nappy, which was then stored in a faecal specimen container. If faeces were too liquid and a specimen was unable to be obtained, plastic film inside the nappy was used to assist sample collection or the whole nappy was collected for analysis. Stool samples were stored at 2–10 °C within 4 h of collection, and transported to the Universitas Gadjah Mada microbiology laboratory within 24 h. Upon receipt at laboratory, stool samples were aliquoted and stored at −70 °C.

2.3. Rotavirus antigen testing

Stool samples were tested for rotavirus antigen using the commercial rotavirus enzyme immunoassay (EIA) ProSpecT (Oxoid, Ltd, UK), as per manufacturer's instructions. Severe rotavirus gastroenteritis was defined as rotavirus gastroenteritis with a modified Vesikari score of ≥11 [8].

2.4. Rotavirus genotyping

Viral RNA was extracted from 10% to 20% w/v faecal extracts of each specimen using the Viral Nucleic Acid Extraction Kit II (Geneaid) according to the manufacturer's instructions. The rotavirus G and P genotype were determined for each sample by the application of independent hemi-nested multiplex reverse transcription polymerase chain reaction (RT-PCR) assays. The first-round RT-PCR assays were performed using the Superscript III One-Step RT-PCR (Invitrogen), using VP7 conserved primers 9Con1-L and VP7R, or VP4 conserved primers Con-2 and Con-3 [16,17]. The second-round genotyping PCR reactions were conducted using specific oligonucleotide primers for G types 1, 2, 3, 4 and 9 or P types [4], [6], [8], [9], [10] [18]. The G and P genotype of each sample was assigned using agarose gel analysis of second-round PCR products.

2.5. Polyacrylamide gel electrophoresis

G3P[8] samples with adequate volume were selected for analysis. The 11 segments of rotavirus dsRNA were separated on 10% (w/v) polyacrylamide gel with 3% (w/v) polyacrylamide stacking gel at 25 mA for 16 h. The genome migration patterns (electropherotypes) were visualized by silver staining according to the established protocol [19,20].

2.6. Amplification of complete rotavirus genomes

The 11 gene segments were reverse transcribed and amplified by PCR using the PrimerScript High Fidelity RT-PCR Kit (Takara, Japan) as previously described [21]. Primers used in the amplification of the 11 gene segments are detailed elsewhere [22].

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