Anti-Rotavirus Protein Reduces Stool Output in Infants With Diarrhea: A Randomized Placebo-Controlled Trial

SHAFIQUL A. SARKER,[1] MARTIN JÄKEL,[2] SHAMIMA SULTANA,[1] NUR H. ALAM,[1] PRADIP K. BARDHAN,[1] MOHAMMOD J. CHISTI,[1] MOHAMMED A. SALAM,[1] WINFRIED THEIS,[2] LENNART HAMMARSTRÖM,[3] and LEON G. J. FRENKEN[2]

[1]International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B), Dhaka, Bangladesh; [2]Unilever R&D Vlaardingen BV, Vlaardingen, The Netherlands; and [3]Division of Clinical Immunology and Transfusion Medicine, Department of Laboratory Medicine, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden

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BACKGROUND & AIMS: Rotavirus infection is a leading cause of morbidity and mortality in children younger than 5 years of age. Current treatment options are limited. We assessed the efficacy of a llama-derived, heavy-chain antibody fragment called anti-rotavirus protein (ARP1), in modifying the severity and duration of diarrhea in male infants with rotavirus infection. **METHODS:** We performed a double-blind, placebo-controlled trial of 176 male infants (6–24 months old) with severe rotavirus-associated diarrhea at Dhaka Hospital, Bangladesh. The infants were randomly assigned to groups given oral ARP1 (15–30 mg/kg/day, n = 88) or placebo (maltodextrin, n = 88) for a maximum of 5 days. The primary outcomes were severity (stool output) and duration of diarrhea and fecal excretion of rotavirus. Secondary outcomes were intake of oral rehydration salt solution, severity of vomiting, and serum levels of rotavirus-specific IgA. **RESULTS:** In infants with only rotavirus infection, total cumulative stool output was 305.47 g/kg body weight among those given placebo (n = 63) and 237.03 g/kg body weight among those given ARP1 (n = 61) (a difference of 68.44 g/kg body weight or 22.5%; 95% confidence interval: 18.27–118.59 g/kg body weight; $P = .0079$). There was a significant reduction in rate of stool output (g/kg/d) in the ARP1 group compared with the placebo group (61%; $P = .002$). ARP1 had no significant effect in infants with concomitant infections or on any other measured outcomes. No adverse events could be linked to ARP1. **CONCLUSIONS: In a placebo-controlled trial, ARP1 reduced stool output in male infants with severe rotavirus-associated diarrhea. Clinicaltrials.gov number: NCT01259765.**

Keywords: Pediatric; Acute Diarrhea; Anti-Rotavirus Intervention; Clinical Study.

Between 450,000 and 500,000 deaths of children younger than 5 years of age can be attributed to rotavirus infection every year. Most of these occur in developing countries[1,2] and the resulting dehydration is the most common cause of death. The economic burden of rotavirus is significant both in developed and developing countries.[3] Vaccines are efficacious in preventing rotavirus infection in many developed[4] and some developing countries.[5,6] However, in sub-Saharan Africa,[7] Malawi, South Africa,[8] Bangladesh, and Vietnam,[9] efficacy remains lower. Possible factors responsible for such diminished efficacy as well as options for improvement have recently been discussed.[10,11] Despite these observations, the World Health Organization still recommends that rotavirus vaccination be included in all national vaccination programs of countries where rotavirus gastroenteritis remains a substantial public health issue.[11]

Current clinical case management strategies for rotavirus-associated diarrhea include prevention of dehydration by oral rehydration salts (ORS) solutions and continual feeding.[12] Zinc supplementation is also recommended by the World Health Organization and United Nations Children's Fund (UNICEF)[13] to reduce severity and duration of diarrhea, although this is not specific to rotavirus-associated diarrhea. A number of treatment approaches specific to rotavirus-associated diarrhea have previously been studied with some success reported with respect to passive immunization approaches,[14–17] probiotics,[18,19] and a number of drugs.[20–25] Until now, however, none of these have been implemented as a treatment for rotavirus-associated diarrhea.

We, and others, have previously reported a method for large-scale production of variable domains of llama heavy-chain antibodies (termed VHH fragments).[26,27] An anti-rotavirus VHH (termed anti-rotavirus protein or ARP1) has previously been shown to bind and neutralize a variety of rotavirus serotypes/genotypes in vitro[28] and to reduce the severity of infection in a mouse pup model.[29]

Following safety studies in healthy adults and children, a proof of concept clinical trial was performed in male infants with rotavirus infection in Bangladesh. The primary objectives of the study were to evaluate orally administered ARP1 for its ability to reduce the severity and duration of diarrhea associated with rotavirus infection.

Abbreviations used in this paper: ARP1, anti-rotavirus protein 1; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; ICDDR, B, International Centre for Diarrhoeal Disease Research, Bangladesh; ORS, oral rehydration salts.

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Methods

Participants and Study Design

We undertook a single-center, randomized, double-blind, placebo-controlled, parallel-group clinical trial to assess the efficacy of ARP1 compared with placebo in male children presenting with acute onset of dehydrating diarrhea. The study took place at the Dhaka Hospital in the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) between June 2007 and September 2008.

The primary end points of the study were changes in total stool output (expressed as g/kg of body weight per day), duration of diarrhea (in hours), and time to fecal clearance of rotavirus (by enzyme-linked immunosorbent assay [ELISA]) in days from randomization to resolution of illness. Secondary end points assessed included intake of ORS solution from randomization to resolution of illness, the number and duration of vomiting episodes during the study intervention and changes in titers of rotavirus specific IgA.

Male children, aged 6−24 months presenting with a history of acute watery diarrhea of 48 hours or less and at least 4 liquid stools during the previous 24 hours, were considered for study enrollment. Children were also assessed for dehydration according to methodology recommended by the World Health Organization and were considered for enrollment when dehydration was apparent. Only male children were considered for enrollment because accurate stool output measurements were required for a primary end point of the study (see Supplementary Material). After obtaining informed consent, the subjects' baseline physical and medical status was assessed against a number of additional inclusion and exclusion criteria (see Supplementary Material). After confirmation of the presence of rotavirus in stool by ELISA[30] and sending stool samples for additional microbiological assessments (see Supplementary Material), subjects were randomized to either the active or placebo intervention. Patients who were enrolled but subsequently identified as having rotavirus infection and the presence of another enteric pathogen remained in the study.

Ethics Statement

The study was approved by the Research and Ethical Review Committees of the ICDDR, B and Wageningen University, The Netherlands (on behalf of the sponsor). The study was performed according to Good Clinical Practice and the Declaration of Helsinki (2000). Written informed consent was obtained from parents or legal guardians of the child before study enrollment.

Procedures

The blinded active study treatment consisted of the active material, ARP preparation, which was a freeze-dried yeast supernatant consisting of approximately 35% ARP1. The composition was further composed of approximately 30% yeast cell protein and peptides, 30% sugars and a small amount of other components including salts and minerals. Treatment dose was calculated on the basis of previously published studies using colostrum or ARP1 in mouse models.[15,29] Based on a minimum body weight of 5 kg, the maximum daily dosage was set at 35 mg/kg/d of ARP1, which corresponds to 100 mg/kg/d of the ARP preparation. To administer the ARP1, single-dose sachets were prepared containing 165 mg of the ARP preparation, 835 mg maltodextrin (Glucidex IT12, Roquette, France), and 5 mg caramel color no. 663 (D. D. Williamson & Co., Louisville, KY). As the actual body weights ranged from 5.69 to 11.29 kg, the administration of 3 sachets per day resulted in an actual dose of 15−30 mg ARP1 per kg body weight per day. The placebo consisted of 1000 mg of maltodextrin with 5 mg caramel color.

Each dose was resuspended in 10 mL ORS solution and administered orally by the nursing staff. The first dose was administered at 4 PM on the day of randomization and every 8 hours after that. Treatments were administered for a minimum of 4 days (if diarrhea resolved) and a maximum of 5 days. If diarrhea continued beyond 5 days, the child was transferred to the general ward of the hospital for further management following the hospital's standard protocol. Resolution of illness was defined as passage of formed or soft stools during 2 consecutive 6-hour periods. Patient follow-up occurred 21 days after first dose.

The nursing staff recorded the frequency, consistency, and amount/volume of stool and urine passed every 6 hours (to a sensitivity of 1 g). This was achieved through a combination of placing the subject on a cholera cot and collecting urine in a pediatric urine collection bag. Data collected from this procedure was also used to calculate amounts of ORS solution required to account for fluid losses due to diarrhea. Stool consistency scores (see Supplementary Material) were used to determine the duration of diarrhea. A small amount of freshly passed stool was obtained daily at 4 PM to determine the presence of rotavirus by ELISA[30] for up to 5 days. These data were used to calculate the duration of viral clearance in stool. ELISA-positive stool samples and serum samples from day 1 were assessed for rotavirus serotype (G and P genotypes) and rotavirus-specific IgA, respectively.

Randomization and Masking

Randomization of subjects was achieved using a 1:1 ratio of active vs placebo treatment in blocks of 4. An independent statistician generated the randomization scheme using R software (R Foundation for Statistical Computing). The master randomization code was kept by the pharmacist for treatment assignment. The investigator and sponsor also kept code envelopes for unblinding in emergency situations and for use in the blind review meeting. The subjects, principal investigator, staff, and laboratory personnel were masked to the treatment assignments. After mixing active or placebo powders with ORS solution, the study interventions were indistinguishable.

Sample Size Calculation

The sample size was estimated on the basis of previous studies,[15,17,31] demonstrating an additional reduction in stool output after administration of bovine, colostrum-derived immunoglobulins in comparison with placebo in rotavirus-induced diarrhea of 30%.[15] In this case, a total of 67 children per group could demonstrate a treatment effect with 5% significance and an 80% power. Based on previous experience,[17] we anticipated that approximately 20% of the children presenting with rotavirus diarrhea would have concomitant infections. A final sample size of 88 subjects per group (176 in total) was selected to account for the latter and an expected 5% dropout rate.

Statistical Analysis

Total mean cumulative stool output per group were compared using Welch's 2-sample t test. Stool output per day was compared via Student's t tests (according to protocol). We subsequently found that the data distribution did not meet the