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The effect of probiotics and zinc supplementation on the immune response to oral rotavirus vaccine: A randomized, factorial design, placebo-controlled study among Indian infants

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ABSTRACT

Background: Strategies are needed to improve oral rotavirus vaccine (RV), which provides suboptimal protection in developing countries. Probiotics and zinc supplementation could improve RV immunogenicity by altering the intestinal microbiota and immune function.

Methods: Infants 5 weeks old living in urban Vellore, India were enrolled in a randomized, double-blind, placebo-controlled trial with a 4-arm factorial design to assess the effects of daily zinc (5 mg), probiotic (10^{10} *Lactobacillus rhamnosus* GG) or placebo on the immunogenicity of two doses of RV (Rotarix®, GlaxoSmithKline Biologicals) given at 6 and 10 weeks of age. Infants were eligible for participation if healthy, available for the study duration and without prior receipt of RV or oral poliovirus vaccine other than the birth dose. The primary outcome was seroconversion to rotavirus at 14 weeks of age based on detection of VP6-specific IgA at ≥20 U/ml in previously seronegative infants or a fourfold rise in concentration.

Results: The study took place during July 2012 to February 2013. 620 infants were randomized equally between study arms and 551 (88.9%) completed per protocol. Seroconversion was recorded in 54/137 (39.4%), 42/136 (30.9%), 40/143 (28.0%), and 37/135 (27.4%) infants receiving (1) probiotic and zinc, (2) probiotic and placebo, (3) placebo and zinc, (4) two placebos. Seroconversion showed a modest improvement among infants receiving probiotic (difference between groups 1, 2 and 3, 4 was 7.5% (97.5% Confidence Interval (CI): −1.4%, 16.2%), p = 0.066) but not zinc (difference between groups 1, 3 and 2, 4 was 4.4% (97.5% CI: −4.4%, 13.2%), p = 0.272). 16 serious adverse events were recorded, none related to study interventions.

Conclusions: Zinc or probiotic supplementation did not significantly improve the low immunogenicity of rotavirus vaccine given to infants in a poor urban community in India. A modest effect of combined supplementation deserves further investigation.

Trial registration: The trial was registered in India (CTRI/2012/05/002677).

1. Introduction

Rotavirus diarrhea is a major cause of infant and child mortality globally, with an especially heavy burden in India [1]. Recent

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estimates put the annual burden in India at 11 million episodes of rotavirus gastroenteritis, resulting in at least USD (2013) $170 million direct costs and 79,000 deaths [2].

Oral rotavirus vaccines, in common with other oral vaccines, have lower immunogenicity and efficacy when given to children in low-income countries compared with high-income countries [3,4]. Experience with the two internationally licensed vaccines – Rotarix and Rotateq – in 36 low-income countries confirms the drop in effectiveness with increasing under-five mortality rate in a country [5]. This lower effectiveness is associated with diminished immunogenicity and potentially a more limited herd-effect [6–8]. Thus, although rotavirus vaccination results in substantial health benefits in low-income countries, reflecting the high burden of disease, these benefits are more limited than if the vaccine were to perform at the levels seen in high-income countries. In India, a newly licensed (since 2014) locally manufactured vaccine (Rotavac) is being introduced to the routine immunization schedule. Seroconversion and efficacy of this vaccine are about 50%, consistent with estimates for Rotarix and Rotateq from low-income countries in Asia and Africa [8–10]. This compares with approximately 90% seen in high-income countries.

The reasons for the diminished performance of oral vaccines in low-income countries are not clearly established, although they may include high levels of maternal antibody, micronutrient deficiencies, early life exposure to enteric pathogens or to the vaccine target, and differences in FUT2 secretor and blood group antigen status [11,12]. Development of practical strategies to enhance the immune response and efficacy of rotavirus vaccines are urgently needed.

The intestinal microbiota is known to play a central role in the development and homeostasis of local mucosal immunity, and may be important in determining the adaptive immune response to live oral vaccination [13]. Additionally, recent work in animal models has highlighted the significance of the microbiota and associated products (e.g. bacterial lipopolysaccharide) for the replication of enteric viruses [14]. In children in low-income countries the intestinal microbiota may be altered because of exposure to a fecally contaminated environment, infection with pathogens, frequent use of antibiotics or because of malnutrition. In addition, environmental enteropathy following repeated exposure to pathogens is common [15]. These changes have been hypothesized to affect the immunogenicity of oral vaccines, although their significance is unclear [16,17]. Probiotics have the potential to change the intestinal microbiota and release bacterial products that interact directly with lymphoid tissue, thereby altering the replication and immune response to oral vaccines [18]. In a study among Finnish infants who received a candidate oral rotavirus vaccine, probiotic (*Lactobacillus* strain GG) administration before and after vaccination resulted in modestly higher serum IgA titers [19]. Studies of other oral vaccines including cholera, typhoid and poliovirus vaccines have had mixed findings [18,20]. We recently reported an increase in IgG antibody responses in children given *Lactobacillus* GG for four weeks following an acute rotavirus infection, indicating that antibody responses to natural infections as well as to vaccines may be influenced by probiotic administration [21].

Zinc deficiency is very common among Indian children [22]. Zinc plays a key role in the functioning of the adaptive immune system, and deficiency is associated with depressed T cell function [23]. Studies have examined the effect of supplementation with zinc on the response to vaccination, including oral poliovirus vaccine (OPV) and inactivated oral cholera vaccine [24–28]. In a study in rural Pakistan, supplementation with 10 mg zinc daily from birth to 18 weeks of age had no impact on seroconversion after 4 doses of trivalent OPV [25]. Zinc supplementation did increase serum vibriocidal antibody titers in children and adults following administration of inactivated oral cholera vaccine, although this effect was not apparent in infants 6–9 months old [26–28]. We are not aware of any studies of zinc supplementation in children and the response to rotavirus vaccine.

Taken together, published studies on probiotic and/or zinc supplementation are insufficient for public health action to improve the efficacy of oral rotavirus vaccines in low-income countries. We therefore investigated their effect on the immune response to oral rotavirus vaccine (Rotarix) in a four-arm placebo-controlled randomized controlled trial with a factorial design among infants in India.

2. Materials and methods

2.1. Study design and participants

Children were randomly assigned (1:1:1:1) in a factorial design to one of four treatment arms: (1) probiotic supplement and zinc supplement, (2) probiotic supplement and zinc placebo, (3) probiotic placebo and zinc supplement, (4) probiotic placebo and zinc placebo, using a blocked randomization procedure with varying block size of 4 and 8 by generated on a computer by an independent statistician. An independent pharmacist packaged test products by subject ID. Study staff assigned sequential subject IDs to eligible consenting participants and remained blinded to assignment throughout the study.

Children were recruited from Chinnallapuram, a densely populated urban area of Vellore, India. Children were eligible for enrolment if they were between 35 and 41 days of age, weighed 3.2 kg or more, available for 11 weeks of follow-up, and had no medical condition that precluded study involvement. Those having received any OPV beyond a birth dose or rotavirus vaccine prior to enrolment were not eligible for participation. Written informed consent was obtained from parents or legal guardians before recruitment.

The Institutional Review Boards (IRB) of the Christian Medical College (CMC), the US Centers for Disease Control and Prevention and the Western IRB (Washington, USA) approved the study protocol. An independent data safety and monitoring board provided study oversight. The trial was registered in the clinical trial registry of India (CTRI/2012/05/002677).

2.2. Interventions

Children received Rotarix (containing $10^{6.5}$ $CCID_{50}$ of the RIX4414 human rotavirus strain; Glaxosmithkline Biologicals) at 6 and 10 weeks of age. Children received other vaccines according to the routine immunization schedule, including BCG at birth, trivalent oral poliovirus vaccine (OPV) at birth, 6, 10 and 14 weeks of age, and pentavalent vaccine against diphtheria, pertussis, hepatitis B and *Hemophilus influenzae* B at 6, 10 and 14 weeks. A birth dose of hepatitis B vaccine was also available in tertiary hospitals with coverage in the study population at this time of about 35%. Routine vaccine doses administered at 6 and 10 weeks were given at the same time as Rotarix by study staff. Each child received daily, a 5 ml suspension containing 5 mg zinc sulphate (Zinc sulphate heptahydrate 1 mg/ml prepared by CMC Pharmacy) or its placebo (CMC pharmacy) and the probiotic strain LGG (*Lactobacillus rhamnosus* GG gelatin capsule with 10^{10} organisms; i-Health Inc, Cromwell, CT) or its placebo (i-Health Inc, Cromwell, CT), from a week before the first dose of rotavirus vaccine at 6 weeks to a week after the second dose at 10 weeks of age. Zinc supplement was administered orally and probiotics by emptying the contents of the gelatin capsule into 5 ml of expressed breast milk before feeding. The dose and timing of these interventions were based on considerations of likely therapeutic effects, safety and past studies of these interventions in infants [21,29].

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