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Stability of live attenuated rotavirus vaccine with selected preservatives and primary containers

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ABSTRACT

Rotavirus infection, which can be prevented by vaccination, is responsible for a high burden of acute gastroenteritis disease in children, especially in low-income countries. An appropriate formulation, packaging, and delivery device for oral rotavirus vaccine has the potential to reduce the manufacturing cost of the vaccine and the logistical impact associated with introduction of a new vaccine, simplify the vaccination procedure, and ensure that the vaccine is safely and accurately delivered to children. Single-dose prefilled presentations can be easy to use; however, they are typically more expensive, can be a bottleneck during production, and occupy a greater volume per dose vis-à-vis supply chain storage and medical waste disposal, which is a challenge in low-resource settings. Multi-dose presentations used thus far have other issues, including increased wastage of vaccine and the need for separate delivery devices. In this study, the goals were to evaluate both the technical feasibility of using preservatives to develop a liquid multi-dose formulation and the primary packaging alternatives for orally delivered, liquid rotavirus vaccines. The feasibility evaluation included evaluation of commonly used preservatives for compatibility with rotavirus vaccines and stability testing of rotavirus vaccine in various primary containers, including Lameplast's plastic tubes, BD's oral dispenser version of Uniject™ (Uniject DP), rommelag's blow-fill-seal containers, and MEDInstill's multi-dose vial and pouch. These presentations were compared to a standard glass vial. The results showed that none of the preservatives tested were compatible with a live attenuated rotavirus vaccine because they had a detrimental effect on the viability of the virus. In the presence of preservatives, vaccine virus titers declined to undetectable levels within 1 month. The vaccine formulation without preservatives maintained a stability profile over 12 months in all primary containers that was similar to its profile in standard glass vials. This study demonstrates that there are multiple options for the primary container for rotavirus vaccines intended for oral delivery. Selection of an optimal primary container should take into consideration additional factors, including stability as well as cold chain volume, usability, cost, and manufacturing feasibility.

1. Introduction

Rotavirus is the leading cause of severe dehydrating diarrhea in children under 5 years of age, accounting for nearly one-third of the estimated 700,000 annual deaths from diarrheal diseases worldwide [1,2]. While most children, even in industrialized countries, become infected with rotaviruses, 90% of the deaths occur in low-income countries [3]. Because there are no effective medications for the infection, the essential treatments for saving lives are intravenous fluids or oral rehydration therapy and zinc supplements, but these often are not available in low-resource settings. Rotaviruses are so contagious and resilient that simple measures such as encouraging hand-washing and providing clean water,

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which are effective in curbing other diarrheal diseases, do not work well. Immunization is the most promising intervention for preventing rotavirus infection. There are two oral, liquid, live attenuated vaccines that are considered safe and effective and are currently available internationally: Rotarix® (GlaxoSmithKline) and RotaTeq® (Merck and Co., Inc.). A number of randomized, controlled trials have shown that both vaccines had 80–90% efficacy against severe rotavirus disease in industrialized countries, and 40–60% efficacy in low-income countries that have high mortality from the disease [3]. Additional vaccines, ROTAVAC® (Bharat Biotech International, Ltd.) and Rotavin (POLYVAC), have recently been licensed for use in India and Vietnam, respectively; however, unlike Rotarix and RotaTeq, these vaccines are not currently prequalified by the World Health Organization (WHO). WHO recommends that rotavirus vaccine for infants be included in all national immunization programs, and Gavi, the Vaccine Alliance, provides support for introduction in eligible low- and lower-middle income countries [4,5]. To date, 79 countries have included rotavirus vaccines in their public-sector immunization regimen, including 36 Gavi-eligible countries [6]. Although, the currently marketed vaccines have been successful, new vaccines are needed to meet rising demand, decrease costs, and achieve greater effectiveness in developing countries. Access to new rotavirus vaccines not only will save children's lives, but also will lessen the tremendous economic and health impact of rotavirus disease. The worldwide economic burden associated with rotavirus disease has been estimated to approach one billion dollars annually [7,8].

Current rotavirus vaccines take up large amounts of space in the cold chain (17 and 46 cm^3 per dose for the most compact, liquid presentations of Rotarix and RotaTeq, respectively) [9,10] due to the fact that they are single dose, consist of comparatively large dose sizes (2 mL) to accommodate antacid to protect the live vaccine in the stomach [11] and many presentations have inefficient packaging.

A potential solution is to provide vaccine in multi-dose containers. Preservatives are commonly used in multi-dose vials of inactivated or subunit liquid injectable vaccines to prevent contamination and extend open-vial shelf life. For example, many vaccines contain thimerosal, and some tetanus–diphtheria–acellular pertussis vaccines and the inactivated poliovirus vaccine contain the preservative 2-phenoxyethanol (2-PE). However, there are no published data on the use of preservatives with live attenuated virus vaccines or oral vaccines, including rotavirus.

The United States National Institutes of Health (NIH) has developed vaccines against the four most common human rotavirus serotypes (G1, G2, G3, and G4), as well as reassortant rotaviruses having G8 (often found in Africa) or G9 (often found in India) serotypes, and licensed them as candidate live attenuated bovine rotavirus vaccines to several manufacturers, mostly based in developing countries, for further development [8]. In this study, we worked with human-bovine reassortant virus serotype G1 (monovalent) formulated vaccine rather than multivalent vaccine, for ease of evaluation of candidate presentations of oral rotavirus vaccine.

In order to assess the feasibility of alternative presentations of rotavirus vaccine that may be more suitable in low-resource settings, we first evaluated several preservatives currently used in marketed parenteral vaccines or in oral medicinal products for infants for possible use in a multi-dose oral rotavirus vaccine presentation. We then investigated primary containers that might minimize the space occupied by rotavirus vaccine in the cold chain. The specific aims of this study were to assess the effect of preservatives on the G1 vaccine formulation and to conduct a 12-month stability study on the optimal vaccine formulation packaged in selected primary containers. For the latter, we evaluated three classes of primary packaging technologies: (1) standard glass vial, (2) single-dose and multi-mono-dose (MMD) prefilled presentations, and (3) novel multi-dose packaging for vaccines.

2. Materials and methods

Frozen bulk monovalent G1 bovine-human reassortant rotavirus was obtained from the Serum Institute of India Private Ltd. and formulated with stabilizing excipients and antacid buffer, using a modified version of a formulation originally developed by Aridis Pharmaceuticals (San Jose, CA, USA) [12]. The PATH rotavirus vaccine formulation contains sucrose, hydrolyzed gelatin, trisodium citrate dehydrate, citric acid, calcium chloride, zinc chloride, and potassium phosphate dibasic. The osmolarity of this formulation is >2000 mOsm. The target viral titer of this vaccine was 5×10^5 fluorescent focus units per milliliter (FFU/mL), observed microscopically (see assay methods), which was equivalent to 1×10^6 FFU in a 2 mL dose. The dilution range in the assay was set at 1:10 to 1:40,960 (fourfold serial dilution; total of seven dilutions). Single-use aliquots (100 μl) of the bulk vaccine were stored at -80 °C and included in each assay run as a positive control for assay quality control.

The preservatives 1% 2-PE (Fluka; St. Louis, MO, USA) and 0.225% methylparaben sodium plus 0.025% propylparaben sodium (parabens) (Fisher Scientific; Waltham, MA, USA) were tested for antibacterial and antifungal efficacy and for their effect on viability of rotavirus. The antibiotic neomycin sulfate (12.5 μg/mL; Sigma, St. Louis, MO, USA) was also evaluated for its effectiveness as a preservative.

A low-passage, mycoplasma-free MA104 host cell line was obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA) for use in viral titer assays. MA104 cells were routinely cultured in Dulbecco's Modified Eagle's Medium (DMEM) (HyClone Laboratories, Inc.; Logan, UT, USA) with 10% FetalClone™ III (derived from fetal calf serum, termed "FCS" herein), 100 U/mL penicillin, and 100 μg/mL streptomycin (Thermo Fisher Scientific; Waltham, MA, USA). Serum-free Advanced DMEM (Gibco; Grand Island, NY, USA) was used for specific assay steps. Trypsin for activating the rotavirus in the G1 vaccine before infecting the MA104 cell monolayers was obtained from Worthington (Lakewood, NJ, USA). Sheep anti-rotavirus polyclonal antibody was from Murdoch Children's Research Institute (Australia) and donkey anti-sheep Alexa Fluor® 488-conjugated secondary antibody was from Invitrogen (Carlsbad, CA, USA). Acetone for fixation of cell monolayers infected with rotavirus was from Sigma. A solution containing 4′,6-diamidino-2-phenylindole (DAPI) from Invitrogen was used to stain the nuclear DNA of MA104 host cells in culture.

For each study, standard 5 mL Type I borosilicate glass vials with silicone rubber stoppers and metal tear-off seals obtained from West Pharmaceutical Services, Inc. (Exton, PA, USA), were used as primary containers. For the 12-month stability study, the G1 vaccine filled into five alternative types of containers was compared with the G1 vaccine in glass vials. Three of the five test containers (see Fig. 1) were prefilled single-dose delivery devices: the blow-fill-seal tube (BFS) (rommelag; Buchs, Switzerland); plastic tubes (Lameplast Group; Bologna, Italy); and Uniject™ DP device (BD; Franklin Lakes, NJ, USA). BFS and Lameplast tubes were obtained as sterile, empty, sealed tubes with a single-piece design (tube and twist-off cap were molded out of the same piece of plastic). The Uniject devices were obtained as sterile, unsealed containers on a reel from BD. The Uniject devices with needles were used for these studies because they were available for testing and had the same vaccine-contacting surfaces as the oral dispenser version (Uniject DP) that would be suitable for oral rotavirus vaccine delivery. The remaining two test containers were a multi-dose vial and multi-dose pouch from Medical Instill Technologies (MEDInstill)

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