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Comparative analysis of oral delivery systems for live rotavirus vaccines

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Abstract

In this paper a comparison of delivery systems for a live rotavirus vaccine is presented. The loss of infectivity was estimated during incorporation into the delivery systems, and during the subsequent processing steps in the preparation of poly(DL-lactide-co-glycolide) microspheres, alginate microcapsules, spray-coated non-pareil seeds, granules, and tablets. Incorporation of live rotavirus into DL-PLG microspheres or alginate microcapsules, as well as the application to the surface of non-pareil seeds resulted in a complete or significant loss of rotavirus infectivity. In contrast, stabilization of the rotavirus vaccine with an excipient blend of cellulose, starch, sucrose and gelatin (30:30:30:10), followed by incorporation into granules or tablets, produced outstanding results with only minimal losses of infectivity. Of these two delivery systems tablets produced better results. However, the dosage form must be modified into a formulation suitable for immunizing infants.

Keywords: Rotavirus; Oral delivery systems; Tableting; Microspheres; Non-pareil seeds; Oral vaccination

1. Introduction

Rotaviruses cause severe, acute diarrhea in young children, and may be responsible for 1–2 million deaths per year worldwide [1–6]. Thus, an effective rotavirus vaccine would significantly reduce global childhood mortality [3]. Infection and reinfection with rotavirus are common in adults [7–9] and, although the symptoms are usually subclinical [10], they contribute to significant loss of productivity in developed countries.

The first successful attempts to immunize infants against rotavirus infection have employed modified live viruses administered by the oral route, and several such vaccines are now in clinical development. Problems remain, however, in the effective administration of these vaccines, particularly in the development of a suitable oral vaccine delivery system. Although the infectivity of rotavirus is relatively stable under various test conditions [11], the virus is acid-sensitive. It begins to lose infectivity at pH 3.5 and the outer capsid of human rotavirus

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collapses below pH 3.0 [12–14]. This acid-sensitivity affects the efficacy of orally-administered vaccines, as the live viruses can be inactivated in the acid environment of the stomach [15]. Gastric acid can be neutralized by the administration of buffers before vaccine administration [12,16,17], but this procedure is time consuming, expensive [18] and poorly tolerated by infants. One approach to this problem is to encapsulate the live rotavirus and to coat with enteric polymers that are stable at acid pH and protect the virus from inactivation, but which dissolve at intestinal pH and release the infectious rotavirus in the gut. However, to our knowledge, no group has demonstrated incorporation of a live rotavirus vaccine into an enteric delivery system with subsequent recovery of significant levels of rotavirus infectivity.

In the present study, we addressed the problem of oral delivery of live rotavirus vaccines by conducting a comparative analysis of retention of infectivity in a variety of solid oral dosage systems developed for use with drugs. Loss of infectivity was estimated during incorporation into the delivery systems, and during the subsequent processing steps in the preparation of poly(DL-lactide-co-glycolide) (DL-PLG) microspheres, alginate microcapsules, granules, tablets and spray-coated non-pareil seeds. These studies indicated that stabilization of the virus was required during incorporation into the delivery system. Tableting was found to maintain the infectivity of the live rotavirus during the initial stages of preparation and during subsequent processing steps.

2. Materials and methods

Porcine rotavirus Gottfried strain GP46 was a gift from Dr. L.J. Saif (Ohio State University, Wooster, OH) and porcine rotavirus strain SB-1A was a gift from Dr. A.Z. Kapikian (NIAID, Bethesda, MD). Pancreatin was purchased from Gibco BRL (Grand Island, NY). Lactose, sucrose, starch, microcrystalline cellulose (Avicel) and gelatin were purchased from Foremost Ingredients (Baraboo, WI); Sigma Chemical (St. Louis, MO); Colorcon (West Point, PA); FMC Corporation (Philadelphia, PA); and Geo A. Hormel (Austin, MN); respectively. Tableting excipients, acdisol, stearic acid and talc were purchased from FMC, Mallinckrodt Specialty Chemical Co. (St. Louis, MO) and Luzenac America Inc. (Englewood, CO). The enteric coating Eudragit L30D, Opadry and cellulose acetate phthalate were purchased from Rohm Pharma (Malden, MA); Colorcon; and Eastman Chemical Company (Kingsport, TN); respectively. Sodium alginate (Keltone HV) was obtained from the Kelco Division of Merck (San Diego, CA) and non-pareil seeds from Paulaur Corporation (Robbinsville, NJ). The Coomassie Blue Binding Assay kit was purchased from Pierce (Rockford, IL).

2.1. Virus growth and purification

Rotavirus was grown in roller bottles on confluent monolayers of MA 104 (fetal rhesus monkey kidney cells) in Dulbecco's modified essential medium (DMEM) supplemented with 30 μg/ml of pancreatin until a cytopathic effect was evident (approximately 2–3 days). The culture media was harvested and clarified by centrifugation at 4°C for 20 min at 3700 × g_{av}, and the virus pelleted by centrifugation at 4°C for 1 h at 130 000 × g_{av}. The pellets were resuspended in DMEM, overlayed on a 30% glycerol-phosphate buffered saline (PBS) cushion, and centrifuged in an SW41 rotor (Beckman, Palo Alto, CA) at 4°C for 4.5 h at 200 000 × g_{av}. The pellets were then suspended in 0.5 ml PBS containing 1% BSA and kept at −80°C until use.

2.2. Plaque assay of rotavirus infectivity

Confluent monolayers of MA 104 cells in six-well plates were washed twice with PBS and inoculated with 0.2 ml of a serial 10-fold dilution of the samples. After absorption for 1 h at 37°C, the unabsorbed virus was removed and the cells overlayed with 4 ml per well of 0.9% agar in DMEM containing 30 μg/ml of pancreatin. After incubation for 5 days at 37°C, 3 ml of a second overlay medium consisting of 0.9% agar in DMEM and 0.03% neutral red was added, and the plaques were counted on the next 2 days. The results are presented as plaque forming units (pfu), or as log loss of pfu from the original dose.

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2.3. Scanning electron microscopy (SEM)

Microspheres/granules were mounted on a specimen disc and coated with a 20 angstrom layer of palladium gold. The coating was carried out using the electron microscope-500 sputter coater. After coating, the samples were examined and photographed using an ISI-SX40 SEM.

2.4. Microencapsulation in DL-PLG microspheres by solvent extraction

Rotavirus-containing microspheres were prepared by the Southern Research Institute (Birmingham, Alabama). Three mg of purified rotavirus strain SB-1A containing approximately 10^{12} pfu of infectious particles was microencapsulated in biodegradable and biocompatible polymers of DL-PLG by a modification of an emulsion-based methylene chloride solvent evaporation procedure described previously [19]. The surface morphology was evaluated by electron microscopy and a smooth surface of continuous polymeric coating was confirmed. The vaccine content (core loading) was estimated by dissolving a sample of the microspheres in methylene chloride, extracting the rotavirus, determining the amount of protein and calculating the percent antigen by weight. The core loading of 0.55% was satisfactory. The size of the microspheres was determined using a Malvern light scattering device and was found to range from 1 to 10 μm.

A sample of microspheres was dissolved in methylene chloride, the virus extracted twice with PBS, and the infectivity measured by plaque assay.

2.5. Microencapsulation in cellulose acetate phthalate (CAP) microspheres by spray drying with the Buchi 190 mini-spray dryer

CAP has been used widely as an enteric coating polymer for pharmaceutical tablets or granules. CAP dissolves at approximately pH 5.5, and thus it withstands prolonged contact with the acid contents of the stomach, but dissolves and releases drugs readily in the small intestine.

The rotavirus strain SB-1A preparation containing approximately 10^8 pfu of infectious particles was resuspended in 100 μl of 1.5 M sucrose containing 10 mM poly-L-lysine and mixed with 40 ml of an aqueous solution of CAP (25 mg/ml, pH 6.5). Microcapsules were produced by atomization of the CAP and vaccine emulsion through a Buchi 190 mini-spray dryer (Brinkmann, Westbury, NY). About 50% of the material was recovered as microcapsules of 1 μm to 5 μm in diameter, as determined by SEM.

The infectivity of the microencapsulated rotavirus was determined by in vitro analysis. Fifty mg of microcapsules were aliquoted into 2 tubes. One sample was treated with 0.1 N HCl at 37°C for 30 min. The microcapsules did not dissolve in the acidic solution and appeared to be intact. The microcapsules were then pelleted by low speed centrifugation and dissolved in 0.5 ml of simulated intestinal fluid, pH 7.5 (USP XXI). The other 50 mg of microcapsules were dissolved in 0.5 ml of simulated intestinal fluid without pretreatment with acid. Both samples were then examined for virus infectivity by plaque assay.

2.6. Incorporation into alginate microcapsules by the process of ionic gelation

Alginate microcapsules containing the live rotavirus strain SB-1A virus were formed by chelation of the sodium alginate with calcium ions. Rotavirus at a concentration of 1×10^8 pfu was mixed with 3 ml of sodium alginate solution (1.2% w/v in normal saline). This suspension was then dripped slowly through a 19 gauge needle into a solution of calcium chloride (1.5% w/v in distilled water) while stirring at 500 revs./min. The microcapsules containing rotavirus were collected by sieving, rinsed three times with a normal saline solution, and then dried at 4°C under vacuum.

Half of the alginate microcapsules containing rotavirus were incubated at 37°C in simulated gastric fluid (USP XXI) for 30 min. They were pelleted and then suspended in phosphate buffered saline (PBS), pH 7.7. The other half of the alginate microcapsules were not exposed to gastric fluid, but were resuspended in PBS, pH 7.7 until dissolved. The rotavirus was released from the alginate microcapsules, which dissolved completely at the higher pH. The rotavirus infectivity of both samples was then determined by plaque assay. The infectivity of the

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live rotavirus was reduced by 2 log after exposure to gastric fluid, but reduced by only 0.73 log when exposed to PBS alone.

2.7. Preparation of granules for oral delivery

Granules were prepared by forming an excipient blend of cellulose, starch, sucrose and gelatin at a ratio of 30:30:30:10 in a Waring blender. One batch of granules was prepared by adding a solution of rotavirus strain SB-1A at a concentration of 4.51×10^7 pfu per 300 mg of excipient blend, and a second batch prepared by adding a solution of SB-1A at a concentration of 3.91×10^4 pfu per 20 mg of excipient blend. The wet mass was dried under vacuum at 4°C and then ground with a small mill to form granules. These were passed through a series of sieves (300 μm–3 mm, USA standard testing sieves), and granules of 1–3 mm were collected and stored at 4°C under vacuum with desiccant.

One batch of granules containing 4.51×10^7 pfu rotavirus strain SB-1A/300 mg of cellulose blend was enterically coated with Eudragit L30D, using a Wurster spray coating method in a laboratory scale fluid bed, STREA-1 (Aeromatic Inc., Columbia, MD). Eudragit is an aqueous dispersion of an anionic copolymer based on methacrylic acid and acrylic acid ethyl ester. The polymer dissolves above pH 5.5 by forming salts with alkalis, thus affording coatings that are insoluble in a gastric media, but soluble in the small intestine. Sufficient coating was applied to increase the weight of the granules by 25% (w/w). Although a weight gain of 12–18% is considered to be protective by manufacturer's standards, the extensive porosity of the granules required a heavier coating to adequately seal the pores.

A second batch of granules containing 3.91×10^4 pfu of rotavirus strain SB-1A per 20 mg cellulose blend was enterically coated with Eudragit L30D as described above, except that granules received a protective coating of Opadry prior to application of the Eudragit. It has previously been determined that enteric polymers can inactivate some ingredients such as viruses, proteins and peptides. Opadry, which is a water-soluble hydoxypropylmethylcellulose-based polymer, was applied to the granules as a protective coating by the Wurster spray coating method in a fluid bed laboratory unit, STREA-1, to give a weight increase to the granules of approximately 8–10%. A further weight gain of 25% occurred on coating with Eudragit.

Uncoated granules, granules coated with Eudragit L30D alone, granules coated with Opadry alone, and granules coated with both Opadry and Eudragit L30D, were stored at 4°C under vacuum with desiccant. The viral infectivity was determined by plaque assay. Disintegration analysis was used to determine the effectiveness of the film coatings in protecting the live rotavirus vaccine from exposure to gastric fluid. The granules were weighed prior to and after exposure to simulated gastric fluid (USP; pH 1.2) at 37°C for 1 h, and the percent gastric uptake determined. The granules then were exposed to simulated intestinal fluid (USP; pH 6.8) at 37°C, and the time required for complete disintegration determined.

2.8. Preparation of tablets

Rotavirus (Gottfried strain GP46) was prepared for tableting using a drying procedure as described for the preparation of the granules except that the rotavirus was dried, (1) with the excipient blend of cellulose, starch, sucrose and gelatin at a concentration of 9.7×10^6 pfu/100 mg of excipient blend, or (2) the rotavirus was dried with lactose alone at a concentration of 6.7×10^4 pfu/20 mg lactose.

Tablets formed from preparation 1 were composed of the following ingredients (% dry weight): lactose filler (74.70%), acdisol disintegrant (3%), stearic acid lubricant (1.50%), talc as a processing aid (1.0%), and the dried rotavirus preparation (19.70%). Tablets formed from preparation 2 were composed as follows: lactose (54.10%), acdisol (3%), stearic acid (1.50%), talc (1.0%), and the dried rotavirus preparation (40.40%). The dry ingredients from each preparation were mixed well to form a dry blend, incorporated into a 3 mm dye and pressed at 550 lb pressure to produce 50 mg, 3 mm tablets.

The tablets were enterically coated with Opadry and Eudragit L30D as described for the coating of the granules. The subcoating of Opadry was applied until the weight of the tablets increased by 6–8%. Subsequently, an enteric coating of Eudragit L30D was applied until the weight of the tablets was

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further increased by 20–25%. Tablets from the three processing steps including uncoated tablets, tablets coated with Opadry, and tablets coated with both Opadry and Eudragit L30D, were stored at 4°C under vacuum with desiccant. The tablets were evaluated for in vitro stability by plaque assay and disintegration testing as described for the granules.

2.9. Comparison of the effects of excipients on the drying process

As infectivity may be lost on drying of the rotavirus during granulation and prior to tableting, the effect of the formulation of the excipient blend on the drying process was evaluated. The formulations included lactose alone, sucrose alone, gelatin alone or the excipient blend of cellulose, starch, sucrose and gelatin at a ratio of 30:30:30:10. The ingredients were suspended in distilled water and mixed with rotavirus strain Gottfried GP46 (1.9×10^7 pfu) until a wet mass was formed. The wet mass was dried in a desiccator at 4°C under vacuum until a level of 5% water weight was reached, then ground with a small mill to form a fine, dry powder. The viral activity retained after processing was determined by plaque assay.

2.10. Application to the surface of non-pareil seeds and enteric coating

As an alternative to granules and tablets, which involve incorporating the live rotavirus inside the core material of the delivery system, the rotavirus was applied to the surface of non-pareil sugar seeds. The dried, live rotavirus strain Gottfried GP46 at a concentration of 10^7-10^9 pfu was suspended in 100 ml of an aqueous hydroxypropylmethylcellulose (HPMC) film-forming polymer that contained 1–2% sucrose. This was applied to the surface of 200 g non-pareil seeds by the Wurster spray coating method administered in a STREA-1 laboratory unit as previously described. Subsequently, a sub-coating of Opadry was applied to an approximate weight gain of 6–8%, followed by an enteric coating of Eudragit L30D that further increased the weight of the seeds by 20%. Seeds from the three processing steps including uncoated seeds, seeds coated with Opadry, and seeds coated with both Opadry and Eudragit L30D, were stored at 4°C under vacuum with desiccant. The seeds were evaluated for in vitro stability by plaque assay and disintegration analysis, as described for the granules.

3. Results

3.1. Comparison of delivery systems

Microencapsulation of live rotavirus indicated that incorporation of live rotavirus into DL-PLG microspheres with solvent removal by either extraction or evaporation, completely destroyed all viral infectivity (Fig. 1). Furthermore, microencapsulation of rotavirus in CAP polymer particles by the process of atomization in a Brinkmann Buchi 190 mini-spray dryer, completely destroyed rotavirus viral infectivity. Incorporation of live rotavirus into alginate microcapsules resulted in a significant loss of infectivity of approximately 2 log after exposure of microcapsules to gastric fluid, but reduced infectivity

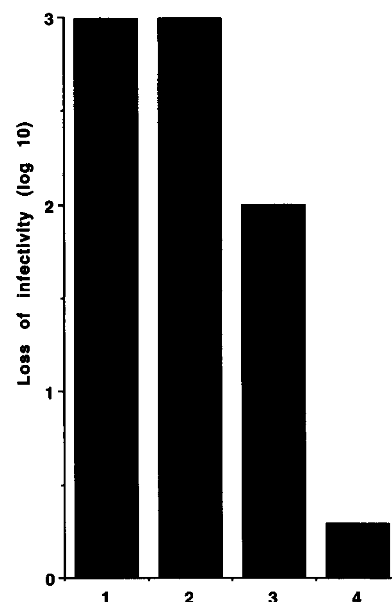

Fig. 1. Loss of rotavirus infectivity after incorporation into various delivery systems. (1) PLGA microspheres; (2) Buchi spray drying; (3) Alginate microcapsules; (4) Cellulose granules. Each bar represents the loss of infectivity during incorporation into these commonly used delivery systems. Cellulose granules show a comparatively lower loss of infectivity.

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