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Original Article

A novel guluronate oligomer improves intestinal transit and survival in cystic fibrosis mice

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Abstract

Background: Cystic fibrosis (CF) patients experience intestinal complications characterized by the accumulation of thick viscous mucus. CF mice were utilized to determine if a novel guluronate oligomer, OligoG, may be a potential therapy in reducing intestinal mucus and subsequent CF-related intestinal manifestations.
Methods: Intestinal transit, intestinal histology, survival and growth were examined in wildtype and CF mice on regular water and OligoG.
Conclusions: OligoG improves intestinal transit and survival in CF mice by reducing the accumulation of intestinal mucus. OligoG's ability to directly bind mucin, disrupt mucin interaction and/or sequester calcium allowing for mucin expansion may explain the decrease in mucus accumulation.

Keywords: Cystic fibrosis; Intestinal transit; Mucus; Genetically modified mouse

1. Introduction

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. The absence of functional CFTR, a cAMP-regulated anion channel, leads to decreased anion and fluid transport resulting in the accumulation of thick sticky mucus within organs including the lung, pancreas and intestine [1,2]. This mucus contributes to intestinal manifestations observed in CF, including slowed transit time, small intestinal bacterial overgrowth (SIBO) and intestinal obstructions [1]. Intestinal obstruction is common in CF patients with 13–20% of patients experiencing meconium ileus (MI) at birth, 10–20% of patients experiencing distal intestinal obstruction syndrome (DIOS) postnatally and half of all patients experiencing constipation throughout life [3]. Common treatment for these obstructions are rehydration, enemas, oral or intestinal osmotic laxatives and, in rare cases, surgery [3,4]. More effective therapy options are necessary for the treatment and prevention of these CF intestinal complications.

The CF mouse displays similar intestinal manifestations as observed in CF patients. The CF mouse displays the accumulation of intestinal mucus, reduced intestinal transit time, SIBO, reduced body weight and a high incidence of intestinal obstruction [5–7]. The CF mouse has been utilized to identify new insights into CF intestinal pathophysiology and to test potential therapies for CF intestinal manifestations [1,8–10]. While the pathophysiology of CF intestinal manifestations is complex, both the accumulation of mucus, due to decreased fluid secretion, and improper mucin expansion are suggested to be primary factors in the CF-related intestinal symptoms [1,11].

Alginates are linear polymers of (1,4) linked-L-guluronate (G) and -D-mannuronate (M) residues that are found as biopolymers in brown algae and bacteria [12]. Alginate is safe for human consumption and administration (US FDA reference

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#21CFR184.1724) and is a commonly used ingredient in foods and medicines [13,14]. The ability to generate these polymers with defined molecular weight and G/M composition may provide new disease therapies. OligoG is a specifically engineered (>85% G) low molecular weight alginate oligomer [15]. OligoG displays antibacterial properties in that it disrupts and inhibits biofilm formation as well as potentiates the action of certain antibiotics through its ability to directly bind to the bacterial surface [15–18]. Additionally, OligoG has been shown to bind mucin directly and disrupt interactions in complex mucus by reducing cross-linking within mucus [19–22]. The current study was undertaken to determine whether OligoG would decrease CF intestinal manifestations in CF mice through its ability to disrupt complex interactions within mucus.

2. Materials and methods

2.1. Mice

The CF mouse model utilized in these experiments contained the well characterized F508del mutation (*Cftr*^{tm1kth}) [7]. The mutation is congenic on the C57BL/6J background and wildtype littermates were used as controls. All mice were allowed unrestricted access to chow (Harlan Teklad 7960; Harlan Teklad Global Diets). Wildtype (WT) and CF mice treated with OligoG (AlgiPharma AS) were allowed access to a 2% OligoG/4% sucrose solution in sterile water (osmolality of 2% OligoG was 45 mosmol/kg). The sucrose was added to make the experimental compound more palatable. OligoG is an alginate oligosaccharide (>85% guluronic acid content, Mw 2600 Da) for which the specific structure and synthesis have previously been published [15,21]. For appropriate controls, WT and CF mice not treated with OligoG were allowed access to 4% sucrose solution in sterile water; however, no differences were observed in WT or CF mice with or without sucrose in the water. For intestinal transit time experiments utilizing 6–8 week old mice, CF mice were allowed access to an osmotic laxative, PEG-3350 with electrolytes (Kremers Urban; osmolality 282 mosmol/kg), and then put on regular water one week before these experiments. All mice were maintained on a 12 h light, 12 h dark cycle at a mean ambient temperature of 22 °C. The Institutional Animal Care and Use Committee of Case Western Reserve University approved all animal protocols.

2.2. Measurement of gastrointestinal transit

Gastrointestinal transit was analyzed as previously described [5]. Briefly, mice were fasted overnight and were allowed free access to water. In the morning mice were given 100 μl of 25 mg/ml rhodamine labeled dextran (Sigma-Aldrich) solution by gavage. 40 min after gavage, the mice were sacrificed and the gastrointestinal tract, from stomach to cecum, was removed and placed in cold saline. The small intestine was divided into 10 equal sections and each segment, in addition to the stomach, was flushed with 2 ml of saline. The flushed contents were centrifuged at 500 rpm for 10 min and 200 μl of the supernatant from each section was placed in a 96 well plate. The quantification of the fluorescent signal in the supernatant from each segment was determined utilizing a multi-well fluorescence plate reader (FLUOstar Omega plate reader; BMG Labtech; excitation 545 nm and emission 590 nm). The distribution of the fluorescent signal in the intestinal segments was used to calculate the geometric center of fluorescence (GCF). GCF was determined by calculating the fraction of fluorescence per segment multiplied by the segment number (1–10) and adding all segments together. GCF can range from 1 to 10 with a higher number indicating a faster intestinal transit time.

2.3. Intestinal histology and mucus measurements

Ileal sections from mice were removed and fixed in methacarn fixative solution (60% methanol, 30% chloroform, and 10% glacial acetic acid) for 4 h, washed in phosphate buffered saline and stored in 70% ethanol until sectioning. The intestines were paraffin embedded, 5 μm thick longitudinal sections were cut, placed on glass slides and stained with fast red and alcian blue. The slides were scanned via a Leica SCN400 slide scanner and images were analyzed via VisiomorphDP software (Visiopharm). To determine mucus coverage between villi, mucus staining (alcian blue) and non-mucus staining (white) were then assigned in the software and pseudo-colored to indicate the call of each pixel by the software. The percentage of the whole area covered by mucus between each villi was calculated as the area of mucus staining divided by the whole area. Histology sections were sampled from each ileum in two distinct regions of at least 100 μm apart.

2.4. Statistical analysis

Results are expressed as the mean +/− SEM. Differences between groups were determined using a two-way ANOVA with post-hoc Tukey test. Kaplan–Meier survival curves were evaluated using a log-rank test. A P value of <0.05 was considered significant.

3. Results

3.1. Intestinal transit time

To examine whether OligoG could be a potential therapy for the prevention of CF intestinal obstructions, intestinal transit was assessed. 6–8 week old wildtype (WT) and CF mice were given either regular water or water containing 2% OligoG for 7 days, after which the intestinal transit time was determined. As shown in Fig. 1, the fluorescently labeled dextran traveled farther in WT mice than CF mice (GCF = 5.92 vs. 2.78; $P < 0.001$) indicating decreased intestinal transit time in CF mice similar to previous studies [5]. Treatment with OligoG shortened intestinal transit time in CF mice (GCF = 6.52 vs. 2.78; $P < 0.001$), with results similar to WT levels. OligoG