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(57) Abstract: The present invention provides therapeutic compositions and methods for treating gastrointestinal diseases and conditions such as diarrhea, for providing rehydration, for correcting electrolyte and fluid imbalances, and/or for improving small intestine function. In one embodiment, the present invention provides a composition formulated for enteral administration, wherein the composition does not contain glucose. In a preferred embodiment, the composition is formulated for administration as an oral rehydration drink.

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DESCRIPTION

MATERIALS AND METHODS FOR TREATING DIARRHEA

CROSS-REFERENCE TO A RELATED APPLICATION

This application claims the benefit of U.S. provisional application Serial No. 61/596,480, filed February 8, 2012, which is incorporated herein by reference in its entirety.

BACKGROUND OF INVENTION

Rotavirus infection is the leading cause of severe diarrheal diseases and dehydration in infants and young children throughout the world. Symptoms of rotavirus infection include watery diarrhea, severe dehydration, fever, and vomiting. Rotavirus infection can also result in jejunal lesions with maximal damage occurring on day three post-inoculation, and in some instances, causing a reduction of villus surface area to 30% to 50% of normal (Rhoads *et al.* (1996) *J. Diarrhoeal Dis. Res.* 14(3):175-181).

The pathophysiological mechanism through which rotavirus induces diarrhea is via the action of an enterotoxin - non-specific protein-4 (NSP4) on small intestine epithelial cells. NSP4 mobilizes intracellular Ca^{2+} in both small and large intestinal crypt epithelia to mimic the secretory effects of the cholinergic agonist carbachol (CCh) in potentiating cAMP-dependent fluid secretion.

Increase in intracellular cAMP ($[cAMP]_i$) and Ca^{2+} ($[Ca^{2+}]_i$ are known to mediate Cl^- and/or HCO_3^- secretion in diarrhea associated with both infective as well as inflammatory conditions (Zhang *et al.* (2007) *J Physiol* 581(3):1221-1233). The osmotic gradient generated by the chloride secretion results in passive movement of water into the intestinal lumen, thereby causing a watery stool. Cl^- secretion with passive water movement occurs in lesser quantity during normal digestion and absorption, which is essential for proper mixing, churning and smooth propulsion through the gut lumen. In a normal absorptive small intestine, there is a fine balance between absorption occurring in the villus cell region and the secretion from the crypt cells. An imbalance resulting from a decreased absorption, increased secretion, or a combined effect can result in diarrhea.

Calcium activated chloride channels (CaCCs) are involved in important physiological processes. Transfection of epithelial cells with specific small interfering RNA against each of

the membrane proteins that are regulated by IL-4 reveals that TMEM16A, a member of a family of putative plasma membrane protein with unknown function, is associated with calcium-dependent chloride current (Caputo *et al.* (2008) *Science* 322(5901):590-594). TMEM16A is widely expressed in mammalian tissues, including tracheal, intestinal, and glandular epithelia, smooth muscle cells, and interstitial cells of Cajal in the gastrointestinal tract (Namkung *et al., J. Biol. Chem.* 286(3):2365-2374).

Luminal glucose absorption by the enterocytes in the small intestine follows secondary active transport (Hediger *et al.* (1994) *Physiol. Rev.* 74(4):993-1026; Wright *et al.* (2004) *Physiology (Bethesda)* 19:370-376). The sodium-glucose transporter (SGLT-1) has a stoichiometry of 2:1, thereby transporting two sodium ions for one glucose molecule across the luminal membrane (Chen *et al.* (1995) *Biophys. J.* 69(6):2405-2414). The tightly coupled sodium glucose transport is driven by the electrochemical gradient of Na^+ formed by Na-K-ATPase activity. The SGLT-1-mediated, electrogenic Na^+ absorption causes solvent drag, thereby leading to passive absorption of water from the lumen.

Maintenance of hydration is a critical element in the treatment of diarrheal diseases including rotavirus-induced diarrhea. Currently, secretory diarrhea is treated with an oral rehydration drink (ORD) - a salt solution containing sodium and a significant amount of glucose and other sugar molecules. Glucose has always been a mainstay in both enteral and parenteral fluids for correcting electrolyte and nutrient absorption defects associated with disease conditions. ORDs are designed to correct the loss of fluids and electrolytes in secretory diarrhea, based on the theory that upon the active, coupled uptake of sodium and glucose in the small intestine, there is a subsequent influx of water that follows the movement of absorbed state.

Although ORDs provide a significant breakthrough in the treatment of cholera and other diarrheal conditions, there is a need to improve its efficiency. Improved formulation is needed due to the poor rate of rehydration provided by existing ORD formulations. The rate of rehydration in diarrheal patients is not in step with the rate of electrolyte loss. The existing ORD formulations have been shown to be ineffective in treating rotavirus-induced diarrhea, while the exact cause for the ineffectiveness remains unknown. Accordingly, a need exists for improved ORD formulations for treatment of diarrhea.

BRIEF SUMMARY

The present invention provides therapeutic compositions and methods for treating gastrointestinal diseases and conditions such as diarrhea, for providing rehydration, for correcting electrolyte and fluid imbalances, and/or for improving small intestine function.

In one embodiment, the present invention provides a composition formulated for enteral administration, wherein the composition does not contain glucose. In a preferred embodiment, the composition is formulated as an oral rehydration drink (ORD). In another preferred embodiment, the composition is in a powder form, and can be reconstituted in water for use as an ORD.

In one embodiment, the composition of the present invention comprises one or more ingredients selected from free amino acids; electrolytes; di-peptides and/or oligo-peptides; vitamins; and optionally, water, therapeutically acceptable carriers, excipients, buffering agents, flavoring agents, colorants, and/or preservatives. In one embodiment, the total osmolarity of the composition is from about 100 mosm to 250 mosm. In one embodiment, the composition has a pH from about 2.9 to 7.3.

In a further embodiment, the present invention provides a treatment comprising administering, via an enteral route, to a subject in need of such treatment, an effective amount of a composition of the invention. The composition can be administered once or multiple times each day. In a preferred embodiment, the composition is administered orally.

In a preferred embodiment, the present invention provides treatment of diarrhea induced by rotavirus infection and/or NSP4. In another preferred embodiment, the present invention results in decreased Cl^- and/or HCO_3^- secretion and/or improved fluid absorption.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows the saturation kinetics for Na^+-coupled glucose and Na^+-coupled 3-O-methylglucose (3-OMG) transport. **(A)** Increasing concentration of lumen glucose results in a concentration-dependent increase in I_{sc}. Nonlinear curve fit with the Michaelis-Menten model for enzyme kinetics shows $V_{max} = 3.3 \pm 0.19$ $\mu eq \cdot h^{-1} \cdot cm^{-2}$ and $K_m = 0.24 \pm 0.06$ mM. **(B)** Increasing lumen concentration of 3-OMG results in a concentration-dependent increase in I_{sc} with a $V_{max} = 1.9 \pm 0.13$ $\mu eq \cdot h^{-1} \cdot cm^{-2}$ and $K_m = 0.22 \pm 0.07$ mM. Increasing concentration of 3-OMG in tissues pre-treated with H-89 results in a significant decrease in $I_{sc,}$ when compared to that of tissues not pre-treated with H-89. **(C)** Addition of increasing

concentrations of 3-OMG in tissues pre-treated with phlorizin showed no response to glucose. The values are obtained from n=6 tissues.

Figure 2 shows unidirectional and net flux of Na^+ (**A**) and Cl^- (**B**). (**A**) Incubation of small intestine tissues with glucose at a concentration of 0, 0.6, or 6 mM results in no significant difference in $J_{ms}Cl^-$. Glucose induces an increase in $J_{sm}Cl^-$ in the small intestine. Specifically, $J_{sm}Cl^-$ is significantly higher in the presence of 0.6 and 6 mM glucose, when compared to that of 0 mM glucose. At 0 mM glucose, significant Cl^- absorption is observed (when compared to Cl^- absorption level at 0.6 mM and 6 mM glucose), while at 0.6 mM and 6 mM glucose, Cl^- secretion is observed. (**B**). At 0 mM glucose, net Na^+ absorption is observed in small intestine tissues. Minimal Na^+ absorption is observed at 0.6 mM glucose, whereas significant Na^+ absorption is observed at 6 mM glucose. Unidirectional fluxes (J_{ms} and J_{sm}) do not show a significant difference at 0, 0.6 or 6mM glucose. The values are obtained from n=8 tissues.

Figure 3 shows effects of glucose and 3-O-methyl-glucose on intracellular cAMP levels in villus, crypt and whole cell fraction of ileum. (**A**) Forskolin treatment significantly increases intracellular cAMP levels in crypt and villus cells in a similar manner. (**B**) Incubation of cells with 8 mM glucose results in a significant increase in the intracellular cAMP levels in villus cells, but not in crypt cells. (**C**) Incubation of the mucosal scraping consisting of both the villus and the crypt epithelial cells with glucose and 3-O-methyl-glucose, respectively, results in a significant increase in intracellular cAMP levels. Incubation of cells with 3-O-methyl-glucose at 6 mM results in a small but significant increase in intracellular cAMP levels. Incubation of cells with different concentrations of glucose produces similar effects on intracellular cAMP levels. Columns represent the mean values and bars show the S.E.M. The values are obtained from n=4 different mice repeated in triplicate. cAMP levels are standardized to protein levels from respective fractions and expressed as pmol (mg protein)$^{-1}$. * $P < 0.001$ compared with group after addition of forskolin or glucose; #$P < 0.01$ comparison between saline treated and glucose treated villus cells. NS = not significant (Bonferroni's multiple comparisons).

Figure 4 shows effects of glucose and 3-O-methyl-glucose on intracellular Ca^{2+} levels in Caco-2 cells. (**A**) Incubation of Caco-2 cells with 0.6 mM glucose results in an increase in fluorescence, when compared to control. Incubation with 6 mM glucose results in a significant increase in fluorescence, when compared to that of control and 0.6 mM glucose.

In cells pre-incubated (for a period of 45 minutes) with 1,2-**b**is(o-**a**mino**p**henoxy)ethane-N,N,N',N'-**t**etra**a**cetic acid) (BAPTA-AM), glucose fails to stimulate any increase in intracellular Ca^{2+} level. Incubation with 3-OMG results in a significantly lower glucose-stimulated increase in intracellular Ca^{2+} levels than that of glucose at similar concentrations. (**B**) Representative trace showing increase in intracellular Ca^{2+} levels stimulated by glucose at a concentration of 0.6mM and 6 mM.

Figure 5 shows results of pH stat experiments showing Cl^--dependent and Cl^--independent HCO_3^- secretion. (**A**) In the absence of glucose, there is a minimal level of Cl^--independent HCO_3^- secretion. In the presence of 6 mM glucose, removal of lumen Cl^- does not result in a significant decrease in HCO_3^- secretion. (**B**) Effect of anion exchange inhibitor and anion channel blocker on HCO_3^- secretion. Experiments are performed in the presence of lumen Cl^-. In the absence of glucose, addition of 100 μM 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS) abolishes HCO_3^- secretion while 10 μM 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) does not have any inhibitory effect on HCO_3^- secretion. In the presence of 6 mM glucose, NPPB, but not DIDS, inhibits HCO_3^- secretion. The values are obtained from n = 6 tissues from different mice. $P < 0.001$.

DETAILED DISCLOSURE

The present invention provides therapeutic compositions and methods for treating gastrointestinal diseases and conditions such as diarrhea, for providing rehydration, for correcting electrolyte and fluid imbalances, and/or for improving small intestine function.

In one embodiment, the present invention provides a composition formulated for enteral administration, wherein the composition does not contain glucose. In a preferred embodiment, the composition is formulated as an oral rehydration drink (ORD). In another preferred embodiment, the composition is in a powder form, and can be reconstituted in water for use as an ORD.

In one embodiment, the composition of the present invention comprises one or more ingredients selected from free amino acids; electrolytes; di-peptides and/or oligo-peptides; vitamins; and optionally, water, therapeutically acceptable carriers, excipients, buffering agents, flavoring agents, colorants, and/or preservatives. In one embodiment, the total osmolarity of the composition is from about 100 mosm to 250 mosm. In one embodiment, the composition has a pH from about 2.9 to 7.3. In one embodiment, the present invention

provides a treatment comprising administering, via an enteral route, to a subject in need of such treatment, an effective amount of a composition of the invention. The composition can be administered once or multiple times each day. In a preferred embodiment, the composition is administered orally.

In a preferred embodiment, the present invention provides treatment of diarrhea induced by rotavirus infection and/or NSP4. In another preferred embodiment, the present invention results in decreased Cl^- and/or HCO_3^- secretion and/or improved fluid absorption.

Induction of Anion Secretion by Glucose

In accordance with the present invention, it has been found that lumen glucose induces net ion secretion in the small intestine. Specifically, glucose induces an active chloride secretion mediated by increased intracellular cAMP and Ca^{2+} levels. Also, net Na^+ transport in the small intestine is absorptive at high glucose concentrations. In addition, glucose results in bicarbonate secretion in the small intestine.

The present inventors have shown that an increase in intracellular cAMP level mediates Cl^- and/or HCO_3^- secretion. The Cl^- and/or HCO_3^- secretion is largely mediated by cystic fibrosis transmembrane conductance regulator (CFTR) ion channels, which have numerous (~ 20) potential serine and threonine phosphorylation sites. Protein kinase A (PKA) and protein kinase C (PKC) are known to activate CFTR anion channels. In patch clamp studies, it has been shown that CFTR channels are inactivated ("run down") quickly unless continuously activated by PKA, signifying the importance of PKA in the activation of CFTR. Consistent with this observation, pre-treatment of small intestine cells with a potent PKA inhibitor H89 results in a significant reduction in glucose-stimulated net increase in *Isc*.

PKA antagonists have been shown to inhibit SGLT1 protein expression following glucose exposure (Dyer *et al.* (2003) *Eur. J. Biochem.* 270(16):3377-3388). CFTR channels are activated by the cAMP-dependent protein kinase (PKA), leading to anion secretion. Glucose-stimulated increase in I_{sc} in the small intestine is partially mediated by CFTR-mediated ion transport.

Glucose as well as PKA agonists (such as cAMP) have been shown to increase the trafficking of SGLT1 to the brush border membrane (Wright *et al.* (1997) *J. Exp. Biol.* 200(Pt 2):287-293; Dyer *et al.* (2003) *Eur. J. Biochem.* 270(16):3377-3388). The decrease in Vmax indicates a total decrease in current, which represents a decrease in glucose transport. The

decrease in Vmax could result from a reduction of the total number of glucose transporter SGTL1, which is mostly found villus epithelial cells. The loss of villus results in a significant loss of available transporter for taking glucose into the cells.

It has been found that incubating enterocytes with glucose increases intracellular cAMP levels. A greater increase in glucose-induced intracellular cAMP level is observed in villus cells than in crypt cells. Incubating enterocytes with forskolin increases intracellular cAMP levels in both crypt and villus cells (Fig 3A). SGLT1-mediated glucose transport occurs primarily in villus cells instead of in crypt cells, as a greater number of SGLT-1 are located in the villus region than in the crypt region (Knickelbein *et al.* (1988) *J. Clin. Invest.* 82(6):2158-2163). Accordingly, increasing glucose concentrations in crypt cells does not result in increased cAMP response (Fig 3B).

Even at low concentration (*e.g.*, 0.6 mM glucose that is approximately half of its V_{max}), lumen glucose induces net anion secretion. At higher concentrations of glucose, sodium absorption is predominant. Increased lumen glucose concentration increases intracellular cAMP and Ca^{2+} levels. Previous studies have shown that K_m for Na^+-coupled glucose transport is in a range of 0.2 to 0.7 mM (Lo & Silverman (1998) *J. Biol. Chem.* 273(45):29341-29351).

The presence of a residual glucose-mediated increase in *Isc* in cells pre-treated with H-89 indicates that PKA independent pathway(s) exist in glucose-induced anion secretion. Electrogenic anion secretion across the small intestine is mediated by ion channels, which can be classified based on their mechanisms of activation, such as activation by cAMP, Ca^{2+}, cell-volume and membrane potential.

It has also been found that lumen glucose induces an increase in intracellular Ca^{2+} levels. Also, the glucose-induced Cl^- secretion is mediated by PKA-dependent as well as PKA-independent pathways. This indicates that, in addition to CFTR, calcium activated chloride channels (CaCCs) also play a role in glucose-induced anion secretion.

In addition, glucose stimulates electrogenic HCO_3^- secretion. Small intestine cells incubated with glucose exhibit higher levels of HCO_3^- secretion in lumen Cl^--containing solution than in lumen Cl^- free solution (Fig 4A & 4B). These results indicate that anion channels mediate HCO_3^- secretion in the presence of glucose. Also, addition of glucose results in a slight decrease in Cl^--HCO_3^- exchange, when compared to cells with no glucose addition. This decrease may be secondary to an increase in intracellular cAMP level with

glucose. This also indicates that glucose induces anion channel-mediated secretion and inhibits electroneutral Cl^--HCO_3^- exchange.

In addition, small intestine cells were incubated with an anion channel blocker (100 mM NPPB) and an anion exchange inhibitor (100 mM DIDS), respectively. There was significant inhibition of glucose-induced, anion channel-mediated HCO_3^- secretion by NPPB (100 mM) (4.2 ± 0.7 vs 7.6 ± 1.5 $mEq.h^{-1}.cm^{-2}$).

In the presence of anion channel inhibitors, residual HCO_3^- secretion is still observed. This indicates that Cl^--HCO_3^- exchange is present in glucose-mediated secretion. This also indicates that an elevated intracellular calcium level could inhibit sodium-hydrogen exchanger 3 (NHE3) activity during normal digestive function as well as in certain disease conditions. This also indicates that SGLT1 plays a dual role in regulating sodium absorption and, at some time, stimulating a secretory and/or an absorptive defect.

The discovery of glucose-induced secretory mechanism can be used in the treatment of gastrointestinal diseases including diarrhea. Patients with acute diarrheal diseases commonly have impaired glucose absorption that occurs in the upper gastrointestinal tract. The presence of unabsorbed carbohydrates can exert an osmotic effect in the bowel, leading to diarrhea. In addition, glucose increases intracellular Ca^{2+} and/or cAMP levels and induces anion secretion. The secretory effects of glucose have been previously understudied or masked by concurrent Na^+-glucose absorption. Also, due to its secretory effects, glucose administration particularly exacerbates gastrointestinal diseases with impaired Na^+-glucose absorption, such as Crohn's disease and irradiation or chemotherapy-induced enteritis that are associated with shortening of the villi and, therefore, extremely compromised absorption.

During rotavirus infection, although there is a predominant glucose-coupled Na^+ absorption via the sodium-dependent glucose cotransporter (SGLT-1) that is primarily expressed in villus cells, there is a significant calcium activated Cl^- secretion via the calcium activated chloride channel (CaCC or TMEM-16a) in the small intestine. In addition, intracellular glucose activates calcium-activated chloride and fluid secretion. Non-structural protein (NSP4) is an entero-toxin produced by rotavirus. It is discovered that glucose and NSP4, when administered together, results in sustained chloride secretion in cells. As a result, the existing ORD formulations that contain a significant amount of glucose further increase the calcium-stimulated chloride secretion, thereby worsening rotavirus-induced diarrhea.

Therapeutic Compositions

In one aspect, the present invention provides therapeutic compositions for treating gastrointestinal diseases and conditions such as diarrhea, for providing rehydration, for correcting electrolyte and fluid imbalances, and/or for improving small intestine function.

In one embodiment, the composition is formulated for enteral administration and does not contain glucose. In a preferred embodiment, the composition is formulated as an oral rehydration drink. In another preferred embodiment, the composition is in a powder form, and can be reconstituted in water for use as an oral rehydration drink.

In a further embodiment, the composition does not contain any substrate of glucose transporters. In a further specific embodiment, the composition does not contain agonists of sodium-dependent glucose cotransporter (SGLT-1) including, but not limited to, glucose analogs (*e.g.*, non-metabolizable glucose agonists for SGLT-1) and other carbohydrates (such as sugars).

Various substrates of SGLT-1 are known in the art including, but not limited to, non-metabolizable glucose analogs such as α-methyl-D-glucopyranoside (AMG), 3-O-methylglucose (3-OMG), deoxy-D-glucose, and α-methyl-D-glucose; and galactose. Substrates of glucose transporters (*e.g.*, SGLT-1) can be selected based on agonist assays as is known in the art. Also, structural modifications of the glucose and other carbohydrates (such as sugars) can be made to obtain substrates of glucose transporters (*e.g.*, SGLT-1).

In one embodiment, the composition does not contain glucose. In a further embodiment, the composition does not contain carbohydrates (such as di-, oligo-, or polysaccharides) or other compounds that can be hydrolyzed into glucose or a substrate of glucose transporters (*e.g.*, SGLT-1).

In one embodiment, the composition comprises, consists essentially of, or consists of, one or more ingredients selected from free amino acids; electrolytes; di-peptides and/or oligo-peptides; vitamins; and optionally, water, therapeutically acceptable carriers, excipients, buffering agents, flavoring agents, colorants, and/or preservatives.

In another alternative embodiment, the composition comprises, consists essentially of, or consists of, one or more ingredients selected from free amino acids; electrolytes; di-peptides and/or oligo-peptides; vitamins; and, optionally, water, therapeutically acceptable carriers, excipients, buffering agents, flavoring agents, colorants, and/or preservatives;

wherein glucose transporters (*e.g.*, SGLT-1) substrates (such as, glucose, glucose analogs) and/or compounds (such as carbohydrates) that can be hydrolyzed into a substrate of glucose transporters (*e.g.*, SGLT-1), if present in the composition, are present in a total concentration of lower than 0.05 mM or any concentration lower than 0.05 mM including, but not limited to, lower than 0.04, 0.03, 0.02, 0.01, 0.008, 0.005, 0.003, 0.001, 0.0005, 0.0003, 0.0001, 10^{-5}, 10^{-6}, or 10^{-7} mM. In on embodiment, the anti-diarrhea composition does not contain sugar. In another embodiment, the anti-diarrhea composition does not contain glucose transporters (*e.g.*, SGLT-1) substrates (such as, glucose, glucose analogs) and/or compounds (such as carbohydrate) that can be hydrolyzed into a substrate of glucose transporters (*e.g.*, SGLT-1).

Amino acids useful for the anti-diarrhea composition of the invention include, but are not limited to, alanine, asparagine, aspartic acid, cysteine, aspartic acid, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, and tyrosine.

In one embodiment, the subject invention provides an anti-diarrhea composition, wherein the composition comprises, consists essentially of, or consists of free amino acids lysine, glycine, threonine, valine, tyrosine, aspartic acid, isoleucine, tryptophan, and serine; and optionally, dipeptides or oligopeptides made of one or more of free amino acids selected from lysine, glycine, threonine, valine, tyrosine, aspartic acid, isoleucine, tryptophan, and serine, therapeutically acceptable carriers, electrolytes, buffering agents, preservatives, and flavoring agents.

In one embodiment, the amino acids contained in the anti-diarrhea composition are in the L-form. In one embodiment, the free amino acids contained in the therapeutic composition can be present in neutral or salt forms.

In one embodiment, the therapeutic composition further comprises one or more electrolytes selected from Na^+, K^+, Ca^{2+}, HCO_3^-, and Cl^-. In one embodiment, the therapeutic composition comprises sodium chloride, sodium bicarbonate, calcium chloride, and/or potassium chloride.

In certain embodiments, each free amino acid can be present at a concentration from 4 mM to 40 mM, or any value therebetween, wherein the total osmolarity of the composition is from about 100 mosm to 250 mosm. The term "consisting essentially of," as used herein, limits the scope of the ingredients and steps to the specified materials or steps and those that do not

materially affect the basic and novel characteristic(s) of the present invention, *e.g.*, compositions and methods for treatment of gastrointestinal diseases and conditions (which, in certain embodiments, being treatment of diarrhea, such as rotavirus-induced diarrhea), for providing rehydration, for correcting electrolyte and fluid imbalances, and/or for improving small intestine function. For instance, by using "consisting essentially of," the therapeutic composition does not contain any unspecified ingredients including, but not limited to, unspecified free amino acids, di-, oligo-, or polypeptides or proteins; mono-, di-, oligo-, or polysaccharides; or carbohydrates that have a direct beneficial or adverse therapeutic effect on treatment of gastrointestinal diseases and conditions (which, in certain embodiments, being treatment of diarrhea, such as rotavirus-induced diarrhea) for providing rehydration, for correcting electrolyte and fluid imbalances, and/or for improving small intestine function.

Also, by using the term "consisting essentially of," the composition may comprise substances that do not have therapeutic effects on treatment of gastrointestinal diseases and conditions (which, in certain embodiments, being treatment of diarrhea, such as rotavirus-induced diarrhea) for providing rehydration, for correcting electrolyte and fluid imbalances, and/or for improving small intestine function; such ingredients include carriers, excipients, flavoring agents, colorants, and preservatives etc that do not affect treatment of gastrointestinal diseases and conditions (which, in one embodiment, being treatment of diarrhea), for providing rehydration, for correcting electrolyte imbalances, and/or for improving small intestine function.

The term "oligopeptide," as used herein, refers to a peptide consisting of three to twenty amino acids.

The term "oligosaccharide," as used herein, refers to a saccharide consisting of three to twenty monosaccharides. The term "carbohydrates," as used herein, refers to compounds having the general formula of $C_n(H_2O)_n$, wherein n is an integer starting from 1; and includes monosaccharaides, disaccharides, oligosaccharides, and polysaccharides.

In one embodiment, the total osmolarity of the composition is from about 100 mosm to 250 mosm, or any value therebetween including, but not limited to, 120 mosm to 220 mosm, 150 mosm to 200 mosm, and 130 mosm to 180 mosm.

In another embodiment, the total osmolarity of the composition is from about 230 mosm to 280 mosm, or any value therebetween. Preferably, the total osmolarity is from about 250 to 260 mosm. In another embodiment, the composition has a total osmolarity that is any value lower than 280 mosm.

In certain embodiments, the composition has a pH from about 2.9 to 7.3, or any value therebetween including, but not limited to, a pH of 3.3 to 6.5, 3.5 to 5.5, and 4.0 to 5.0.

In certain embodiments, the composition has a pH from about 7.1 to 7.9, or any value therebetween. Preferably, the composition has a pH from about 7.3 to 7.5, more preferably, about 7.2 to 7.4, or more preferably, about 7.2.

In certain embodiments, the composition does not contain one or more ingredients selected from oligo- or polysaccharides or carbohydrates; oligo- or polypeptides or proteins; lipids; small-, medium-, and/or long-chain fatty acids; and/or food containing one or more above-mentioned nutrients.

Treatment of Gastrointestinal Diseases and Conditions

Another aspect of the present invention provides methods for treatment of gastrointestinal diseases and conditions. In certain embodiments, the present invention can be used to treat diarrhea, to provide rehydration, to correct electrolyte and fluid imbalances, and/or to improve small intestine function. In a preferred embodiment, the present invention provides treatment of rotavirus-induced diarrhea. In another preferred embodiment, the present invention provides treatment of diarrhea induced by NSP4.

In one embodiment, the method comprises administering, via an enteral route, to a subject in need of such treatment, an effective amount of a composition of the invention. The composition can be administered once or multiple times each day. In one embodiment, the composition is administered orally.

In a preferred embodiment, the present invention provides decreased Cl^- and/or HCO_3^- secretion and/or improved fluid absorption.

The term "treatment" or any grammatical variation thereof (*e.g.*, treat, treating, and treatment *etc.*), as used herein, includes but is not limited to, alleviating or ameliorating a symptom of a disease or condition; and/or reducing the severity of a disease or condition. In certain embodiments, treatment includes one or more of the following: alleviating or ameliorating diarrhea, reducing the severity of diarrhea, reducing the duration of diarrhea, promoting intestinal healing, providing rehydration, correcting electrolyte imbalances, improving small intestine mucosal healing, and increasing villus height in a subject having diarrhea.

The term "effective amount," as used herein, refers to an amount that is capable of treating or ameliorating a disease or condition or otherwise capable of producing an intended therapeutic effect.

The term "subject" or "patient," as used herein, describes an organism, including mammals such as primates, to which treatment with the compositions according to the present invention can be provided. Mammalian species that can benefit from the disclosed methods of treatment include, but are not limited to, apes, chimpanzees, orangutans, humans, monkeys; domesticated animals such as dogs, cats; live stocks such as horses, cattle, pigs, sheep, goats, chickens; and other animals such as mice, rats, guinea pigs, and hamsters.

In one embodiment, the human subject is an infant of less than one year old, or of any age younger than one year old, such as 10 months old, 6 months old, and 4 months old. In another embodiment, the human subject is a child of less than five years old, or of any age younger than five years old, such as four years old, three years old, and two years old. In one embodiment, the subject in need of treatment of the present invention is suffering from diarrhea.

In one embodiment, the present invention can be used to treat diarrhea. In certain embodiments, the present invention can be used to treat diarrhea caused by pathogenic infections including, but not limited to, infections by viruses, including, but not limited to, rotavirus, Norwalk virus, cytomegalovirus, and hepatitis; bacteria including, but not limited to, campylobacter, salmonella, shigella, *Vibrio cholerae*, and *Escherichia coli*; parasites including, but not limited to, *Giardia lamblia* and cryptosporidium. In a preferred embodiment, the present invention can be used to treat rotavirus-induced diarrhea.

In certain embodiments, the present invention can be used to treat diarrhea caused by injury to the small intestine caused by, for example, infection, toxins, chemicals, alcohol, inflammation, autoimmune diseases, cancer, chemo-, radiation, proton therapy, and gastrointestinal surgery.

In certain embodiments, the present invention can be used in the treatment of diarrhea caused by diseases including, but not limited to, inflammatory bowel diseases (IBD) including Crohn's disease and ulcerative colitis; irritable bowel syndrome (IBS); autoimmune enteropathy; enterocolitis; and celiac diseases.

In certain embodiments, the present invention can be used in the treatment of diarrhea caused by gastrointestinal surgery; gastrointestinal resection; small intestinal transplant; post-surgical trauma; and radiation-, chemo-, and proton therpy-induced enteritis.

In another embodiment, the present invention can be used to treat alcohol-related diarrhea. In another embodiment, the present invention can be used to treat traveler's diarrhea and/or diarrhea caused by food poisoning.

In certain embodiments, the present invention can be used in the treatment of diarrhea caused by injury to the small intestine mucosa, for example, diarrheal conditions in which there is a reduced villous height, decreased mucosal surface areas in the small intestine, and villus atrophy, *e.g.*, partial or complete wasting away of the villous region and brush border. In certain embodiments, the present invention can be used in the treatment of diarrhea caused by injury to small intestine mucosal epithelial cells, including the mucosa layer of duodenum, jejunum, and ileum.

In one embodiment, the present invention can be used to treat secretory diarrhea. In certain embodiments, the present invention can be used to treat secretory diarrhea mediated via the CFTR channels and/or CaCC channels (*e.g.*, TMEM-16a). In one embodiment, the present invention can be used to treat acute and/or chronic diarrhea.

In one embodiment, the present invention can be used to treat diarrhea caused by malabsorption of nutrients. In one embodiment, the present invention can be used to treat secretory diarrhea caused by reduced level or functional activity of glucose transporters such as SGLT-1.

As used herein, the term "diarrhea" refers to a condition in which three or more unformed, loose or watery stools occur within a 24-hour period. "Acute diarrhea" refers to diarrheal conditions that last no more than four weeks. "Chronic diarrhea" refers to diarrheal conditions that last more than four weeks.

In one embodiment, the present invention does not involve the administration of one or more of the following ingredients selected from glucose, glucose analogs, substrates of glucose transporters (*e.g.*, SGLT-1), di-, oligo-, or polysaccharides; carbohydrates; or molecules that can be hydrolyzed into glucose or a substrate of glucose transporters (*e.g.*, SGLT-1).

In certain alternative embodiments, the present invention comprises administering one or more ingredients selected from glucose; glucose analogs; substrates of glucose transporters

(*e.g.*, SGLT-1); di-, oligo-, or polysaccharides; carbohydrates; or molecules that can be hydrolyzed into glucose or a substrate of glucose transporters (*e.g.*, SGLT-1), wherein the total concentration of these ingredients is lower than 0.05 mM or any concentration lower than 0.05 mM including, but not limited to, lower than 0.04, 0.03, 0.02, 0.01, 0.008, 0.005, 0.003, 0.001, 0.0005, 0.0003, 0.0001, 10^{-5}, 10^{-6}, or 10^{-7} mM.

Formulations and Administration

The present invention provides for therapeutic or pharmaceutical compositions comprising a therapeutically effective amount of the subject composition and, optionally, a pharmaceutically acceptable carrier. Such pharmaceutical carriers can be sterile liquids, such as water. The therapeutic composition can also comprise excipients, flavoring agents, colorants, and preservatives etc that do not affect treatment of gastrointestinal diseases and conditions (which, in one embodiment, being treatment of diarrhea), for providing rehydration, for correcting electrolyte and fluid imbalances, and/or for improving small intestine function.

In an embodiment, the therapeutic composition and all ingredients contained therein are sterile. In certain preferred embodiments, the composition is formulated as a drink, or the composition is in a powder form and can be reconstituted in water for use as a drink.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions contain a therapeutically effective amount of the therapeutic composition, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the enteral mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients, *e.g.*, compound, carrier, or the pharmaceutical compositions of the invention. The ingredients of the composition can be packaged separately or can be mixed together. The kit can further comprise instructions for administering the composition to a patient.

Materials and Methods

Animal preparation

Normally fed, 8-week–old, male NIH Swiss mice are sacrificed by CO_2 inhalation, followed by cervical dislocation. The small intestine is gently removed, and the segment is washed and flushed in ice-cold Ringer's solution. Then the mucosa is separated from the serosa and the muscular layers by striping through the submucosal plane as previously described (Zhang *et al.* (2007) *J Physiol* 581(3):1221-1233). Following exsanguinations, ileal mucosa is obtained from a 10 cm segment close to the caecum. All experiments are approved by the University of Florida Institutional Animal Care and Use Committee.

Bio-electric measurements

Ion transport studies are performed on ileal sheets. Tissues are then mounted in between the two halves of an Ussing type-Lucite chamber with $0.3cm^2$ exposed surface areas (P2304, Physiologic Instruments, San Diego, CA, USA). Regular Ringer's solution (115mM NaCl, 25mM $NaHCO_3$, 4.8mM K_2HPO_4, 2.4mM KH_2PO_4, 1.2mM $MgCl_2$ and 1.2mM $CaCl_2$) bubbled with 95% O_2: 5%CO_2 is used bilaterally as bathing solution for the tissues and the temperature is maintained constant at 37^0C. The chambers are balanced to eliminate osmotic and hydrostatic forces. Resistance due to fluid is also compensated. The tissues are allowed to stabilize. The basal short-circuit current (I_{sc}) and the corresponding conductance (G) are recorded using a computer controlled voltage/current clamp device (VCC MC-8, Physiologic Instruments).

Flux studies

Isotope of Sodium, ^{22}Na, is used to study Na flux across the mucosa under basal conditions followed by addition of glucose. Conductance–paired tissues are designated to study serosal to mucosal flux (J_{sm}) representing secretory function, and mucosal to serosal flux (J_{ms}) representing absorptive function. ^{22}Na is added in to the designated side of the tissue and 500µl samples are collected every 15 minutes from the other side. In a separate set of tissues ^{36}Cl is added to either the serosal or the mucosal side. Glucose of 8mM concentration is added into the chamber for full stimulation, and the corresponding changes in I_{sc} and conductance are recorded. Conductance is recorded based on the Ohm's law.

Three samples are collected under each condition. Radioactvity is counted using gamma counter. Tissues with conductance less than 10% change are matched and the average $J_{net} = J_{ms} - J_{sm}$ is calculated.

Protein Kinase A (PKA) inhibitor studies

Tissues paired with similar conductance and current are treated with or without 100μM H-89 (Santa Cruz Biotechnology, Inc, Santa Cruz, CA), an irreversible protein kinase A (PKA) inhibitor. The tissues are incubated with H-89 for 30 minutes. Increasing concentrations of glucose (0.015 – 8mM) are added every 5 minutes and the peak current is noted. Saturation kinetic constant is calculated for the corresponding K_m and V_{max} for treated and untreated tissues.

Caco-2 Cell Culture

Caco-2 cells differentiate post-confluence into cells with functional characteristics of fetal ileal epithelium. Caco-2 cells produce microvilli and have increased expression of small intestine specific transport proteins including SGLT1 and are therefore widely used as a model system for studying enterocyte function.

Caco-2 cells are obtained from ATTC and cultured in Dulbecoo's modified Eagle's medium supplemented with 10% fetal calf serum (FCS) and 1% nonessential amino acids at 37°C and 5% CO_2. Caco-2 cells are passaged for 20-25 times and are seeded (2×10^5 cells/dish) on 5 cm petri-dishes and grown until 80% confluence, when the FCS concentration is changed to 5%. Cells are grown for another 10 days before they are used for functional studies.

Confocal Ca^{2+} fluorescence microscopy

Caco2 cells grown in 25 mm round coverslips are mounted on the bath chamber RC-21BR attached to series 20 stage adapter (Warner Instruments, CT USA). The cells are maintained at 37°C using a single channel table top heater controller (TC-324B, Warner Instruments, CT USA). Cells are loaded with the fluorescent calcium indicator Fluo-8 AM dye (Cat # 0203, TEFLab, Inc., Austin, TX USA) at 0.5 μM concentration at room temperature and incubated for 45 minutes. Confocal laser scanning microscopy is performed using an inverted Fluoview 1000 IX81 microscope (Olympus, Tokyo, Japan) and a U Plan S-Apo 20× objective. Fluorescence is recorded by argon lasers with excitation at 488 nm and emission at 515 nm. The Fluorescent images are collected with scanning confocal microscope. Solutions of either Ringer, glucose-containing Ringer's or BAPTA-AM-containing glucose-Ringer's solution are added to the bath using a multi-valve perfusion

system (VC-8, Warner instruments, Hamden CT, USA) controlled using a VC-8 valve controller (Warner instruments, Hamden CT, USA). Changes are recorded and fluorescence is measured for various cells. Cells are washed with Ringer's solution and the experiment is repeated with the use of 3-O-methylglucose and carbechol (positive control).

Colorimetric cAMP measurements

Freshly isolated mucosal scrapings of ileal epithelial cells are washed three times in Ringer's solution containing 1.2 mM Ca^{2+} at 37°C. Washed cells are then divided into two groups and treated with either saline or 6 mM glucose and incubated for 45 minutes. Cells are treated with 0.1 M HCl to stop endogenous phosphodiesterase activity. The lysates are then used for cAMP assay using cAMP direct immunoassay kit (Calbiochem, USA).

The quantitative assay of cAMP uses a polyclonal antibody to cAMP that binds to cAMP in samples in a competitive manner. After a simultaneous incubation at room temperature, the excess reagents are washed away and substrates are added. After a short incubation time, the reaction is stopped and the yellow color generated is read at 405 nm. The intensity of the color is inversely proportional to the concentration of cAMP in standards and samples. cAMP levels are standardized to protein levels from respective fractions and expressed in pmol (mg protein)$^{-1}$.

Forskolin treated cells are used as a positive control. Glucose and forskolin treated cells are incubated for 45 minutes. All the assays are performed in triplicate and repeated until n = 4 different mice.

EXAMPLES

Following are examples which illustrate procedures and embodiments for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

EXAMPLE 1 - GLUCOSE-STIMULATED INCREASE IN I_{SC} IN ILEUM

This Example shows that glucose stimulates an increase in I_{sc} in mouse ileum. Specifically, addition of glucose (8 mM) to the lumen side results in a significant increase in I_{sc} when compared to its basal level (3.4 ± 0.2 vs 1.1 ± 0.1 μEq.h^{-1}.cm^{-2}). The I_{sc} obtained

using standard Ussing chamber studies is a summation of net ion movement across the epithelium ($I_{sc} = J_{net}Na^+ + J_{net}Cl^- + J_{net}HCO_3^- - J_{net}K^+$).

There are no known Na^+ absorptive (ENaC-mediated) or Na^+ secretory mechanisms in the small intestine. Treatment of the mucosal side of the small intestine with 10 μM amiloride, an epithelial sodium channel inhibitor, produces no effect on I_{sc}.

Therefore, the basal I_{sc} of 1.1 ± 0.1 $\mu Eq.h^{-1}.cm^{-2}$ is primarily due to cystic fibrosis transmembrane conductance regulator (CFTR) activity from the crypt and K^+ secretory current.

To determine the saturation kinetics of Na^+-coupled glucose transport, increasing concentrations of glucose up to 8 mM are added to the lumen side in the presence of 140 mM Na^+. Increasing concentrations of glucose results in an enhanced but saturable rate of I_{sc} (Fig. 1A), with a K_m of 0.24 ± 0.03 mM and a V_{max} of 3.6 ± 0.19 $\mu eq \cdot h^{-1} \cdot cm^{-2}$ for glucose. At glucose concentrations ranging from 0.5 to 0.7 mM, the glucose saturation kinetics show early signs of saturation; nevertheless, continued increase in glucose concentrations results in continued increase in I_{sc}, thereby yielding a knick in the glucose saturation curve at glucose concentrations of 0.5 to 0.7 mM.

EXAMPLE 2 - 3-O-METHYL-GLUCOSE-STIMULATED INCREASE IN I_{SC}

This Example investigates whether the glucose saturation kinetics observed in Example 1 are due to SGLT1-mediated transport but not due to glucose metabolism in the epithelial cells. Specifically, 3-O-methyl-glucose (3-OMG), a poorly metabolized form of glucose, is added to the lumen side to study saturation kinetics of Na^+-coupled glucose transport.

Figure 1B shows the saturation kinetics of 3-OMG, with a V_{max} of 2.3 ± 0.13 $\mu eq \cdot h^{-1} \cdot cm^{-2}$ and a K_m of 0.22 ± 0.07 mM). Addition of 3-OMG results in a significant decrease in V_{max} (2.3 ± 0.13 $\mu eq \cdot h^{-1} \cdot cm^{-2}$ vs 3.4 ± 0.2 $\mu eq \cdot h^{-1} \cdot cm^{-2}$) with no change in K_m in the Na^+-coupled glucose transport, when compared to that with glucose. Similar to glucose, a knick is observed with 3-OMG at concentrations 0.5 to 0.7 mM (Fig. 1B).

EXAMPLE 3 - GLUCOSE-STIMULATED I_{SC} IN THE PRESENCE OF H-89

Based on the currently-known transport mechanisms, the glucose-stimulated increase in I_{sc} could result from electrogenic anion secretion or electrogenic Na^+ absorption.

Protein Kinase A (PKA), also known as the cAMP-dependent protein kinase, is required in the activation of CFTR channels. To study the role for PKA in glucose-induced increase in I_{sc}, tissues are mounted in Ussing chambers and incubated with H-89, a PKA inhibitor, for 45 minutes. Subsequently, the tissues are used for studying glucose saturation kinetics.

In the presence of H-89, glucose shows a V_{max} of 0. 8 ± 0.06 $\mu Eq.cm^{-2}.h^{-1}$ and a K_m of 0.58 ± 0.08 mM. The knick in the glucose saturation curve (observed when ileal tissues are incubated with glucose at concentrations ranging from 0.5 to 0.7 mM) disappears altogether when ileal cells are pre-treated with H-89, with a shift of the saturation curve to the right (Fig. 1C). The results indicate the inhibition of PKA-dependent transport processes at low concentrations of glucose.

Similar to the glucose saturation curve, 3-OMG also shows a PKA-sensitive current. The 3-OMG saturation curve (with H-89 incubation) is not significantly different from that observed with glucose (with H-89 incubation) (Fig 1A & B).

Table 1 Changes in glucose and 3-O-methly-glucose saturation kinetics in the presence and absence of H-89 – a PKA inhibitor.

	V_{max}	K_m	V_{max}	K_m
PKA Inhibitors	-	-	H-89	H-89
Glucose	3.6 ± 0.2	0.2 ± 0.1	1.6 ± 0.1	0.5 ± 0.1
3-OMG	2.7 ± 0.1	0.2 ± 0.1	1.4 ± 0.1	0.6 ± 0.1

* Part of glucose and 3-OMG-stimulated current is abolished in the presence of PKA. Results are from n= 8 tissues.

The results indicate that the PKA-inhibitable current (shown in Table 1) results from the Na^+-coupled glucose transport, instead of from other intracellular metabolisms involving glucose (Table 1).

PKA plays a significant role in cAMP-mediated anion secretion and SGLT1-mediated

Na^+ and glucose absorption. The presence of H-89-insensitive current indicates that glucose stimulates non-PKA-mediated anion secretion (such as intracellular Ca^{2+}-mediated secretion).

EXAMPLE 4 – ABOLISHMENT OF GLUCOSE-STIMULATED INCREASE IN I_{SC} IN THE PRESENCE OF PHLORIZIN

To investigate whether inhibition of glucose transport abolishes PKA-sensitive current, experiments are conducted using phlorizin (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA), a reversible competitive inhibitor of SGLT1. Specifically, ileal tissues mounted in Ussing chamber are treated with 100 μM phlorizin on the lumen side and glucose saturation kinetic studies are conducted.

The results show that glucose-stimulated and /or 3-OMG increase in I_{sc} is completely abolished in the presence of phlorizin (Fig 1C). The results indicate that glucose transporter activity via SGLT1 is essential for the PKA-sensitive and insensitive current.

EXAMPLE 5 – EFFECT OF GLUCOSE ON UNIDIRECTIONAL AND NET FLUX OF SODIUM

Isotopic flux measurements of Na^+ are performed using ^{22}Na at a steady-state rate of transfer from either mucosa to serosa J_{ms} or serosa to mucosa J_{sm}. Net flux of Na^+ is calculated using the equation: $J_{net} = J_{ms} - J_{sm}$. + J_{net} indicates net absorption; whereas -J_{net} indicates net secretion.

In the absence of glucose (0 mM), small intestinal tissues show net sodium absorption (1.8 ± 0.3 $mEq.h^{-1}.cm^{-2}$). Na^+ absorption is abolished in the presence of 0.6 mM glucose. However, addition of 6 mM glucose results in a significant increase in *Jnet* Na^+ (3.2 ± 0.5 $\mu Eq.h^{-1}.cm^{-2}$), indicating net sodium absorption. Unidirectional Na^+ fluxes do not show significant difference at 0, 0.6 and 6 mM glucose (Fig 2B).

EXAMPLE 6 – EFFECT OF GLUCOSE ON UNIDIRECTIONAL AND NET FLUX OF CHLORIDE

Change in I_{sc} at 0.6 mM glucose is calculated as 1.1 $\mu Eq.h^{-1}.cm^{-2}$ (2.2 ± 0.3 - 1.1 ± 0.1 $\mu Eq.h^{-1}.cm^{-2}$) and change in I_{sc} at 6 mM glucose is calculated as 2.2 $\mu Eq.h^{-1}.cm^{-2}$ (3.4 ± 0.2 - 1.1 ± 0.1 $\mu Eq.h^{-1}.cm^{-2}$). The increase in I_{sc} with increasing glucose concentrations cannot be fully explained based on the $J_{net}Na^+$ values (based on values at 0.6 and 6 mM glucose).

Isotopic flux measurements for Cl^- are performed using ^{36}Cl to determine whether Cl^- flux accounts for a portion of the I_{sc} that cannot be attributed to $J_{net}Na^+$. $J_{net}Cl^-$ calculated in the absence of glucose shows Cl^- absorption (2 ± 0.3 $\mu Eq.h^{-1}.cm^{-2}$). The level of sodium absorption (1.8 ± 0.3 $\mu Eq.h^{-1}.cm^{-2}$) is comparable to that of chloride (2.0 ± 0.3 $\mu Eq.h^{-1}.cm^{-2}$) in the absence of glucose, indicating electroneutral Na^+ and Cl^- absorption.

Addition of 0.6 mM or 6 mM glucose to the mucosa side results in net Cl^- secretion (Fig 2A). $J_{net}Cl^-$ at 0.6 mM glucose (-3.6 ± 0.8 $\mu Eq.h^{-1}.cm^{-2}$) and 6 mM glucose (-4.0 ± 1.4 $\mu Eq.h^{-1}.cm^{-2}$) are not significantly different.

The results show that there is a significant increase in $J_{sm}Cl^-$ in the presence of glucose (at 0.6 and 6 mM glucose) ($J_{sm}Cl^-$ 16.9 ± 0.7 $\mu Eq.h^{-1}.cm^{-2}$ and 17 ± 0.7 $\mu Eq.h^{-1}.cm^{-2}$, respectively), when compared to $J_{sm}Cl^-$ in the absence of glucose (11.9 ± 0.4 $\mu Eq.h^{-1}.cm^{-2}$) (Fig 2A). The results indicate that significant Cl^- secretion occurs at a glucose concentration as low as 0.6 mM. Increasing glucose concentration does not result in increased Cl^- secretion.

EXAMPLE 7 – HCO_3^- SECRETION IN ILEUM IN THE ABSENCE OF LUMEN GLUCOSE

Transepithelial electrical measurements and flux studies show that addition of glucose to ileal tissues induces significant Cl^--secretion. While $J_{net}Cl^-$ at 0.6 and 6 mM glucose shows significant anion secretion, this does not account for all of the changes in I_{sc}, especially in view of the significant differences between I_{sc} values at 6 mM glucose 6 $\mu Eq.h^{-1}.cm^{-2}$ (7.5 ± 0.4 - 1.5 ± 0.1 $\mu Eq.h^{-1}.cm^{-2}$) and 0.6 mM.

pH stat studies are performed to determine whether HCO_3^- secretion contributes to the unaccounted portion of the I_{sc}. At least two modes of HCO_3^- secretion in the mouse small intestine have been identified by the present inventors: 1) Cl^--dependent, electroneutral Cl^--HCO_3^- exchange, and 2) Cl^--independent, electrogenic HCO_3^- secretion.

The results show that endogenous HCO_3^- secretion does not contribute to net HCO_3^- secretion. Specifically, HCO_3^--free, poorly buffered solution is added to both sides of the tissues mounted in an Ussing chamber and both sides of the tissues are bubbled with 100% O_2. Minimal HCO_3^- secretion (0.1 ± 0.01 $mEq.h^{-1}.cm^{-2}$, n=12) is recorded under such conditions. Subsequent addition of HCO_3^--containing buffered solution to the basolateral side and bubbling with 95% O_2 and 5% CO_2 on that side results in significant HCO_3^- secretion 3.8 ± 0.2 $mEq.h^{-1}.cm^{-2}$ (n =9).

To determine whether lumen Cl^--independent HCO_3^- secretion plays a role in HCO_3^- secretion (in the absence of lumen glucose), pH stat experiments are performed in the absence of lumen Cl^-. In the absence of lumen Cl^-, minimal HCO_3^- secretion is recorded (0.4 ± 0.1 $\mu Eq.h^{-1}.cm^{-2}$) (Fig 5A). The results indicate that the basal HCO_3^- secretion in the absence of lumen glucose is primarily due to Cl^--dependent, electroneutral Cl^--HCO_3^- exchange.

EXAMPLE 8 – EFFECT OF LUMEN GLUCOSE ON HCO_3^- SECRETION IN ILEUM

pH stat experiments are performed to determine the effect of glucose on lumen Cl^--dependent HCO_3^- secretion. In the presence of lumen Cl^-, addition of glucose to the lumen side results in a significant HCO_3^- secretion (7.6 ± $\mu Eq.h^{-1}.cm^{-2}$).

The HCO_3^- secretion in the presence of glucose could be due to a lumen Cl^--dependent, electroneutral Cl-HCO_3^- exchange or a lumen Cl^--independent anion channel-mediated HCO_3^- secretion. To assess the mechanism of glucose-stimulated HCO_3^- secretion, glucose is added to the mucosal side. Removal of lumen Cl^- does not abolish HCO_3^- secretion in tissues incubated with 6 mM glucose (3.2 ± 0.6 $\mu Eq.h^{-1}.cm^{-2}$) (Fig 5A). The results indicate that HCO_3^- secretion in the presence of glucose is primarily due to lumen Cl^--independent secretion, and is anion channel-mediated.

In another experiment, 100 μM 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB), a non-specific anion channel blocker, is added to the lumen side. NPPB inhibits lumen Cl^--independent HCO_3^- secretion detected in the presence of 6 mM glucose (Fig 5B). The results indicate that glucose-stimulated HCO_3^- secretion is mediated via an anion channel.

To investigate whether glucose-induced HCO_3^- secretion occurs via a CFTR channel, 100 μM glibenclamide is added to the lumen side. Glibenclamide inhibits lumen Cl^--

independent HCO_3^- secretion-stimulated by glucose, indicating that CFTR channels mediate glucose-stimulated HCO_3^- secretion.

EXAMPLE 9 – EFFECT OF GLUCOSE METABOLISM ON ANION CHANNEL-MEDIATED HCO_3^- SECRETION

To assess whether glucose metabolism in the small intestine tissue attributes to the glucose-stimulated HCO_3^- secretion, small intestine tissues are incubated with 3-OMG, a poorly metabolized form of glucose, in the absence of lumen and bath HCO_3^-. HCO_3^- secretion (0.1 ± 0.03 $\mu Eq.h^{-1}.cm^{-2}$) is observed in the presence of 3-OMG (6 mM) and absence of lumen and bath HCO_3^-.

EXAMPLE 10 – EFFECT OF GLUCOSE ON INTRACELLULAR cAMP LEVEL IN ILEUM

In the absence of glucose, cell lysates from the villus cells show a higher intracellular cAMP level, when compared to that of crypt cells. Incubation with forskolin results in a significant increase in $[cAMP]_i$ level in villus and crypt cells (Fig 3A). Forskolin-treated cells are used as a positive control.

To study the effect of glucose on intracellular cAMP levels, the villus and crypt cells are incubated with 6 mM glucose. Incubation of villus cell lysates with glucose results in a significant increase in intracellular cAMP level, when compared to that of crypt cells (Fig 3B). The results indicate that the glucose-mediated increase in intracellular cAMP level plays a role in mediating glucose-stimulated anion secretion. Increased $[cAMP]_i$ is observed in villus cells but not in crypt cells; this indicates that glucose transport machinery is only needed in fully mature and differentiated villus epithelial cells.

To determine whether glucose metabolism has an effect on intracellular cAMP level, mucosal scraping from the ileum is pre-incubated with 3-OMG for 45 minutes and then the cell lysates are used for measuring intracellular cAMP level.

Similar to glucose, incubation of villus cells with 3-OMG at concentrations of 0.6 and 6 mM results in significant increase in intracellular cAMP level (Fig 3C). Incubation of villus cells with 3-OMG at 6 mM results in a significantly higher intracellular cAMP level, when compared to that of 6 mM glucose ($P < 0.01$) (Fig 3C). The results show that the

observed increase in intracellular cAMP level is not caused by glucose metabolism in small intestine tissues.

EXAMPLE 11 – EFFECT OF GLUCOSE ON INTRACELLULAR CA^{2+} IN Caco2 CELL LINES

PKA inhibitor (H-89) inhibits both cAMP-stimulated anion secretion and SGLT1-mediated glucose transport. Presence of H-89-insensitive I_{sc} (Fig 1A & B) indicates that PKA-independent mechanisms also contribute to the glucose-induced secretion. As cAMP, intracellular Ca^{2+} is one of the chief intracellular second messengers involved in anion secretion.

To determine the role of intracellular Ca^{2+} in glucose-stimulated increase in *Isc*, intracellular Ca^{2+} level is measured in the presence of different concentrations of glucose and 3-OMG, respectively, and in the presence of BAPTA-AM (1,2-**b**is(o-**a**mino**p**henoxy)ethane-N,N,N',N'-**t**etra**a**cetic acid) - an intracellular calcium-specific chelator. The Ca^{2+} responses to glucose and 3-OMG in cultured Caco2 cells loaded with the $Ca2^{+}$ indicator fluo 8 are monitored by laser scanning confocal microscopy. Addition of 0.6 mM glucose to the bath medium initiates intracellular Ca^{2+} oscillation (Fig 4 B). The amplitude of the oscillations decreases with time. The mean peak amplitude of calcium fluorescence (F/Fo) with 0.6 mM glucose is calculated to be 1.32 ± 0.1 (n=10).

Glucose-induced Ca^{2+} oscillation is not related to the intracellular metabolism of glucose, as 0.6 mM 3-OMG glucose induces similar Ca^{2+} oscillation (1.2 ± 0.1 (n=10) (Fig 4A). Glucose-stimulated Ca^{2+} oscillation is abolished by pre-incubating the cells with intracellular Ca^{2+} chelator BAPTA-AM for 45 minutes ($1 .01 \pm 0.1$) (n=10) (Fig 4A).

Glucose is added at a higher concentration (6 mM) to determine whether increased glucose concentration increases the amplitude of the Ca^{2+} oscillation. The Ca^{2+} oscillations are significantly higher with addition of glucose (1.85 ± 0.2 vs 1.32 ± 0.1) or 3-OMG (1.5 ± 0.1 vs 1.2 ± 0.2) at 6 mM to the bathing medium, when compared to that of 0.6 mM glucose or 3-OMG (Fig 4A). Glucose-stimulated increase in Ca^{2+} oscillations is completely abolished by pre-incubating the cells with BAPTA-AM (Fig 4A). This indicates that intracellular Ca^{2+} is involved in glucose-induced anion secretion.

EXAMPLE 12 – THERAPEUTIC COMPOSITIONS FOR TREATMENT OF DIARRHEA

In certain embodiments, this Example provides formulations for treating diarrhea, such as rotavirus-induced diarrhea. In one embodiment, the formulation does not comprise glucose, glucose analogs, substrates of glucose transporters, or sugar molecules.

Formulation 1 (Serving Size 1 bottle (237 ml)		
	Amount per serving	% Daily Value*
L-Valine	276 mg *	
L-Aspartic Acid	252 mg *	
L-Serine	248 mg *	
L-Isoleucine	248 mg *	
L-Threonine	225 mg *	
L-Lysine HCL	172 mg *	
L-Glycine	141 mg *	
L-Tyrosine	51 mg *	
Other Ingredients: Water, Electrolytes		

Formulation 2 (Serving Size 1 bottle (237 ml)	
Amount per serving	
	% Daily Value *
Total Fat 0 g	0%
Sodium 440 mg	18%
Total Carbohydrate 0 g	0%
Protein 2g	
Ingredients: Water, Amino Acids (L-Tryptophan, L-Valine, L-Aspartic Acid, L-Serine, L-Isoleucine, L-Threonine, L-Lysine Hydrochloride, L-Glycine, L-Tyrosine), Electrolytes	
Amino Acid	Amount mg/1 bottle serving (237 ml)
L-Lysine HCI	175
L-Aspartic Acid	255
L-Glycine	144
L-Isoleucine	251
L-Threonine	228
L-Tyrosine	52
L-Valine	281
L-Tryptophan	392
L-Serine	252

All references, including publications, patent applications and patents, cited herein are hereby incorporated by reference to the same extent as if each reference was individually and specifically indicated to be incorporated by reference and was set forth in its entirety herein.

The terms "a" and "an" and "the" and similar referents as used in the context of describing the invention are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. Unless otherwise stated, all exact values provided herein are representative of corresponding approximate values (*e.g.*, all exact exemplary values provided with respect to a particular factor or measurement can be considered to also provide a corresponding approximate measurement, modified by "about," where appropriate).

The use of any and all examples, or exemplary language (*e.g.*, "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise indicated. No language in the specification should be construed as indicating any element is essential to the practice of the invention unless as much is explicitly stated.

The description herein of any aspect or embodiment of the invention using terms such as "comprising", "having", "including" or "containing" with reference to an element or elements is intended to provide support for a similar aspect or embodiment of the invention that "consists of", "consists essentially of", or "substantially comprises" that particular element or elements, unless otherwise stated or clearly contradicted by context (*e.g.*, a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

REFERENCES

Zhang H, Ameen N, Melvin JE, & Vidyasagar S (2007) Acute inflammation alters bicarbonate transport in mouse ileum. *J Physiol* 581(3):1221-1233.

Hediger MA & Rhoads DB (1994) Molecular physiology of sodium-glucose cotransporters. (Translated from eng) *Physiol. Rev.* 74(4):993-1026 (in eng).

Wright EM, Loo DD, Hirayama BA, & Turk E (2004) Surprising versatility of Na+-glucose cotransporters: SLC5. (Translated from eng) *Physiology (Bethesda)* 19:370-376 (in eng).

Chen XZ, Coady MJ, Jackson F, Berteloot A, & Lapointe JY (1995) Thermodynamic determination of the Na+: glucose coupling ratio for the human SGLT1 cotransporter. (Translated from eng) *Biophys. J.* 69(6):2405-2414 (in eng).

Torres-Pinedo R, Rivera CL, & Fernandez S (1966) Studies on infant diarrhea. II. Absorption of glucose and net fluxes of water and sodium chloride in a segment of the jejunum. (Translated from eng) *J. Clin. Invest.* 45(12):1916-1922 (in eng).

Davidson GP, Gall DG, Petric M, Butler DG, & Hamilton JR (1977) Human rotavirus enteritis induced in conventional piglets. Intestinal structure and transport. (Translated from eng) *J. Clin. Invest.* 60(6):1402-1409 (in eng).

Nalin DR, *et al.* (1979) Oral rehydration and maintenance of children with rotavirus and bacterial diarrhoeas. (Translated from eng) *Bull. World Health Organ.* 57(3):453-459 (in eng).

Lorrot M & Vasseur M (2007) How do the rotavirus NSP4 and bacterial enterotoxins lead differently to diarrhea? (Translated from eng) *Virol J* 4:31 (in eng).

Chapman MJ, *et al.* (2009) Glucose absorption and gastric emptying in critical illness. (Translated from eng) *Crit Care* 13(4):R140 (in eng).

Telch J, *et al.* (1981) Intestinal glucose transport in acute viral enteritis in piglets. (Translated from eng) *Clin Sci (Lond)* 61(1):29-34 (in eng).

Lo B & Silverman M (1998) Cysteine scanning mutagenesis of the segment between putative transmembrane helices IV and V of the high affinity Na+/Glucose cotransporter SGLT1. Evidence that this region participates in the Na+ and voltage dependence of the transporter. (Translated from eng) *J. Biol. Chem.* 273(45):29341-29351 (in eng).

Wright EM, Hirsch JR, Loo DD, & Zampighi GA (1997) Regulation of Na+/glucose cotransporters. (Translated from eng) *J. Exp. Biol.* 200(Pt 2):287-293 (in eng).

Dyer J, Vayro S, King TP, & Shirazi-Beechey SP (2003) Glucose sensing in the intestinal epithelium. (Translated from eng) *Eur. J. Biochem.* 270(16):3377-3388 (in eng).

Gribble FM (2008) RD Lawrence Lecture 2008: Targeting GLP-1 release as a potential strategy for the therapy of Type 2 diabetes. (Translated from eng) *Diabet. Med.* 25(8):889-894 (in eng).

Gustavsson N, *et al.* (Synaptotagmin-7 as a positive regulator of glucose-induced glucagon-like peptide-1 secretion in mice. (Translated from eng) *Diabetologia* 54(7):1824-1830 (in eng).

Eissele R, *et al.* (1992) Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. (Translated from eng) *Eur. J. Clin. Invest.* 22(4):283-291 (in eng).

Elliott RM, *et al.* (1993) Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. (Translated from eng) *J. Endocrinol.* 138(1):159-166 (in eng).

Herrmann C, *et al.* (1995) Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. (Translated from eng) *Digestion* 56(2):117-126 (in eng).

Knickelbein RG, Aronson PS, & Dobbins JW (1988) Membrane distribution of sodium-hydrogen and chloride-bicarbonate exchangers in crypt and villus cell membranes from rabbit ileum. *J. Clin. Invest.* 82(6):2158-2163.

Vidyasagar S, Rajendran VM, & Binder HJ (2004) Three distinct mechanisms of HCO3-secretion in rat distal colon. *Am J Physiol Cell Physiol* 287(3):C612-621.

Coon S, Kekuda R, Saha P, & Sundaram U (Reciprocal regulation of the primary sodium absorptive pathways in rat intestinal epithelial cells. (Translated from eng) *Am J Physiol Cell Physiol* 300(3):C496-505 (in eng).

Hu Z, *et al.* (2006) MAPKAPK-2 is a critical signaling intermediate in NHE3 activation following Na+-glucose cotransport. (Translated from eng) *J. Biol. Chem.* 281(34):24247-24253 (in eng).

Turner JR & Black ED (2001) NHE3-dependent cytoplasmic alkalinization is triggered by Na(+)-glucose cotransport in intestinal epithelia. (Translated from eng) *Am J Physiol Cell Physiol* 281(5):C1533-1541 (in eng).

Donowitz M & Li X (2007) Regulatory binding partners and complexes of NHE3. (Translated from eng) *Physiol. Rev.* 87(3):825-872 (in eng).

Zachos NC, Kovbasnjuk O, & Donowitz M (2009) Regulation of intestinal electroneutral sodium absorption and the brush border Na+/H+ exchanger by intracellular calcium. (Translated from eng) *Ann. N. Y. Acad. Sci.* 1165:240-248 (in eng).

Zachos NC, *et al.* (2009) NHERF3 (PDZK1) contributes to basal and calcium inhibition of NHE3 activity in Caco-2BBe cells. (Translated from eng) *J. Biol. Chem.* 284(35):23708-23718 (in eng).

Donowitz M, *et al.* (2009) NHE3 regulatory complexes. (Translated from eng) *J. Exp. Biol.* 212(Pt 11):1638-1646 (in eng).

Zhu X, *et al.* (Elevated Calcium Acutely Regulates Dynamic Interactions of NHERF2 and NHE3 Proteins in Opossum Kidney (OK) Cell Microvilli. (Translated from eng) *J. Biol. Chem.* 286(40):34486-34496 (in eng).

Caputo A, *et al.* (2008) TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. (Translated from eng) *Science* 322(5901):590-594 (in eng).

Namkung W, Phuan PW, & Verkman AS (TMEM16A inhibitors reveal TMEM16A as a minor component of calcium-activated chloride channel conductance in airway and intestinal epithelial cells. (Translated from eng) *J. Biol. Chem.* 286(3):2365-2374 (in eng).

Engle MJ, Goetz GS, & Alpers DH (1998) Caco-2 cells express a combination of colonocyte and enterocyte phenotypes. (Translated from eng) *J. Cell. Physiol.* 174(3):362-369 (in eng).

Ball JM, Tian P, Zeng CQ, Morris AP, & Estes MK (1996) Age-dependent diarrhea induced by a rotaviral nonstructural glycoprotein. (Translated from eng) *Science* 272(5258):101-104 (in eng).

Rhoads JM, *et al.* (1996) Can a super oral rehydration solution stimulate intestinal repair in acute viral enteritis? (Translated from eng) *J. Diarrhoeal Dis. Res.* 14(3):175-181 (in eng).

Claims

We claim:

1. A sterile therapeutic composition for treating diarrhea, wherein the composition is formulated for enteral administration and has a total osmolarity from 100 mosm to 250 mosm,

wherein the composition comprises:

one or more free amino acids and/or electrolytes, and water;

and wherein a substrate of a glucose transporter and/or a compound that can be hydrolyzed into a substrate of a glucose transporter, if present in said composition, is present at a concentration of less than 0.01 mM.

2. The composition according to claim 1, wherein the composition does not contain glucose or a glucose analog.

3. The composition according to claim 2, wherein the composition does not contain α-methyl-D-glucopyranoside (AMG), 3-O-methylglucose (3-OMG), deoxy-D-glucose, or α-methyl-D-glucose.

4. The composition according to claim 1, wherein the composition does not contain any carbohydrate.

5. The composition according to claim 1, having a pH of 2.9 to 7.3.

6. A method for treating a subject having diarrhea, wherein the method comprises administering to the subject, via enteral administration, a composition of claim 1.

7. The method according to claim 6, wherein the subject has rotavirus-induced diarrhea.

8. The method according to claim 6, wherein the subject is a human.

9. The method according to claim 8, wherein the subject is five years old or younger.

10. The method according to claim 6, wherein the composition is administered orally.

11. The method according to claim 6, wherein the composition does not contain glucose or a glucose analog.

12. The method according to claim 11, wherein the composition does not contain α-methyl-D-glucopyranoside (AMG), 3-O-methylglucose (3-OMG), deoxy-D-glucose, or α-methyl-D-glucose.

13. The method according to claim 6, wherein the composition does not contain any carbohydrate.

14. The method according to claim 6, wherein the composition comprises one or more free amino acids selected from lysine, glycine, threonine, valine, tyrosine, aspartic acid, isoleucine, tryptophan, and serine.

15. The method according to claim 14, wherein the composition further comprises one or more electrolytes selected from Na^+, K^+, HCO_3^-, CO_3^{2-}, and Cl^-.

16. The method according to claim 6, wherein the composition consists essentially of one or more free amino acids selected from alanine, asparagine, aspartic acid, cysteine, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, proline, glutamine, arginine, serine, threonine, valine, tryptophan, and tyrosine; one or more electrolytes selected from Na^+, K^+, HCO_3^-, CO_3^{2-}, and Cl^-; water; and, optionally, one or more carriers, buffering agents, preservatives, and/or flavoring agents.

17. The method according to claim 6, wherein the composition consists essentially of one or more free amino acids selected from lysine, glycine, threonine, valine, tyrosine, aspartic acid, isoleucine, tryptophan, and serine; one or more electrolytes selected from Na^+, K^+, HCO_3^-, Ca^{2+}, and Cl^-; water; and, optionally, one or more carriers, buffering agents, preservatives, and/or flavoring agents.

18. A package containing the composition of claim 1, or a powder which, when combined with a specified amount of water, makes a composition of claim 1.

19. The package according to claim 18, which is in a powder form which, when combined with water, makes a composition of claim 1.

20. The package acccording to claim 18, further comprising instructions for administering the composition to a subject who has diarrhea.

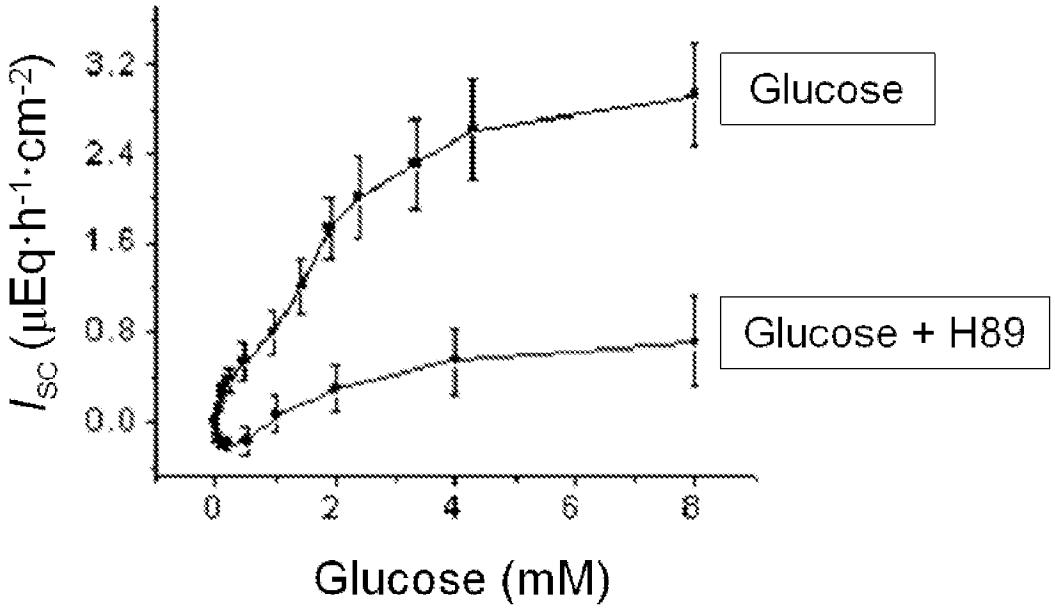
I_{SC} (μEq·h^{-1}·cm^{-2})
3.2
2.4
1.6
0.8
0.0
0
2
4
6
8
Glucose
Glucose + H89
Glucose (mM)

FIG. 1A

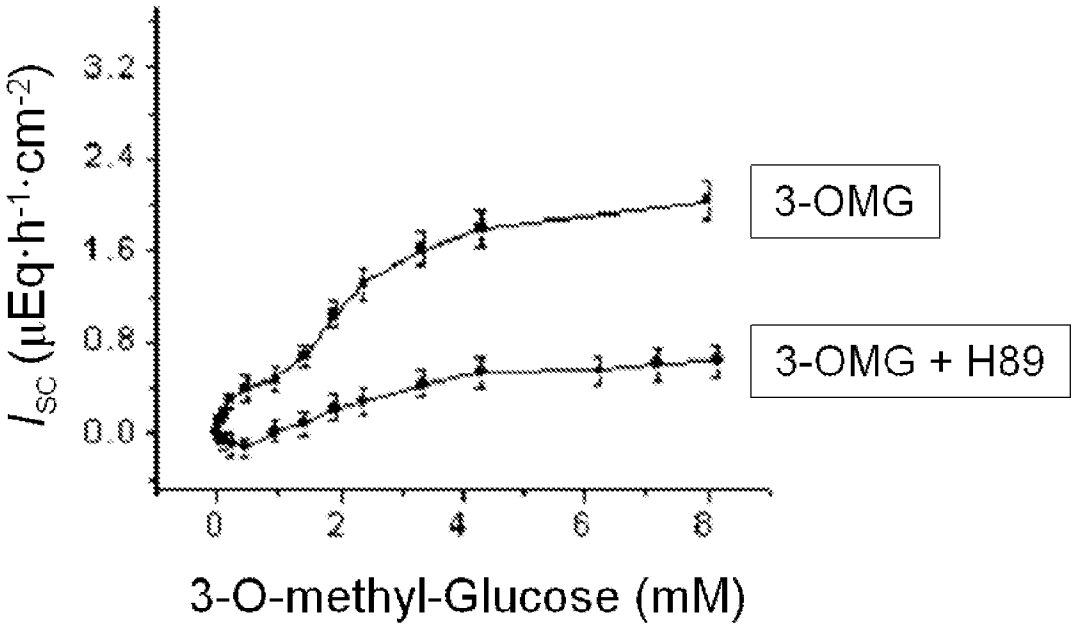
I_{SC} (μEq·h^{-1}·cm^{-2})
3.2
2.4
1.6
0.8
0.0
0
2
4
6
8
3-OMG
3-OMG + H89
3-O-methyl-Glucose (mM)

FIG. 1B

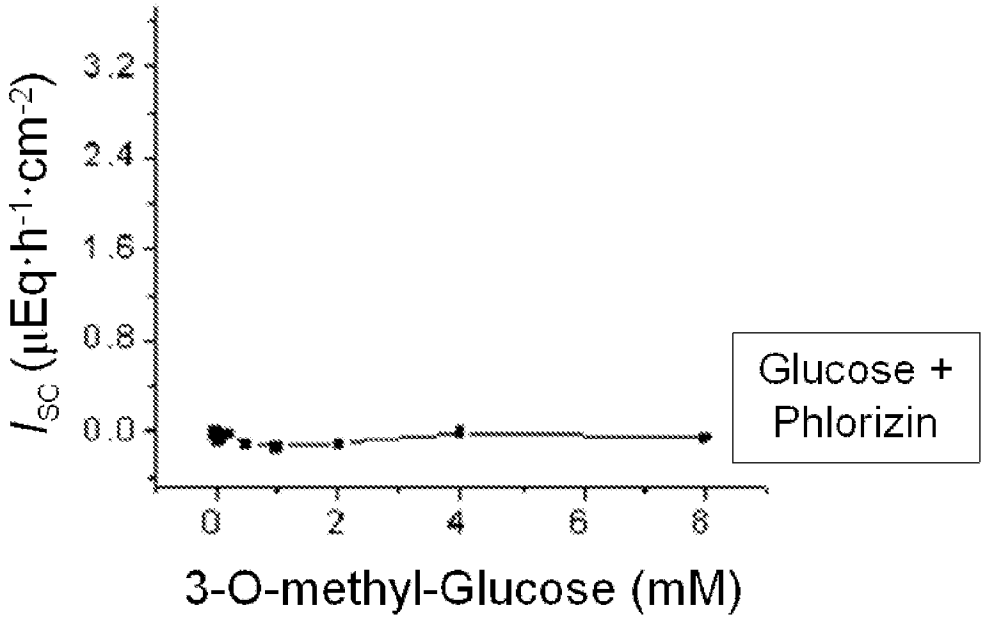
I_{SC} (μEq·h^{-1}·cm^{-2})
3.2
2.4
1.6
0.8
0.0
0
2
4
6
8
Glucose +
Phlorizin
3-O-methyl-Glucose (mM)

FIG. 1C

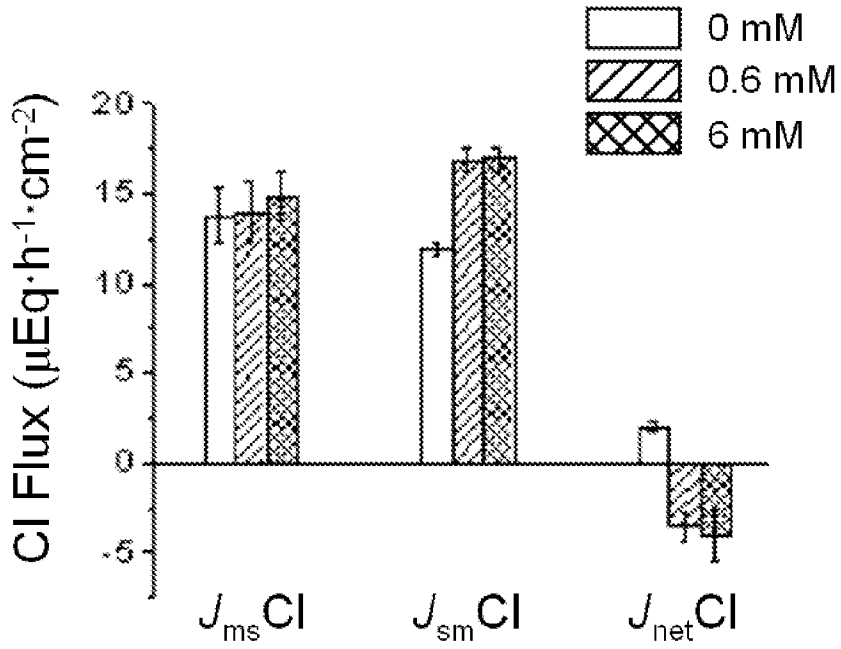
0 mM
0.6 mM
6 mM
Cl Flux (μEq·h^{-1}·cm^{-2})
20
15
10
5
0
-5
J_{ms}Cl
J_{sm}Cl
J_{net}Cl

FIG. 2A

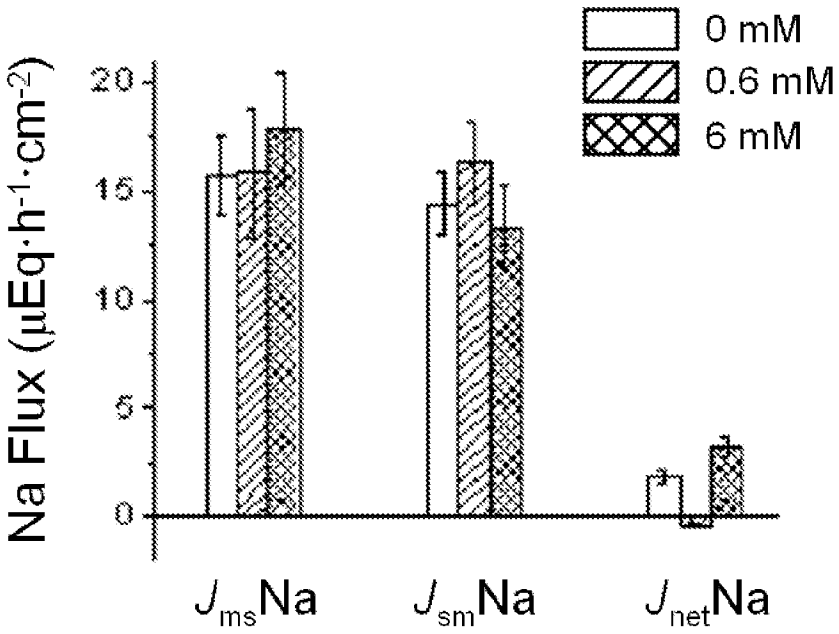
0 mM
0.6 mM
6 mM
Na Flux (μEq·h-1·cm-2)
20
15
10
5
0
Jms Na
Jsm Na
Jnet Na

FIG. 2B

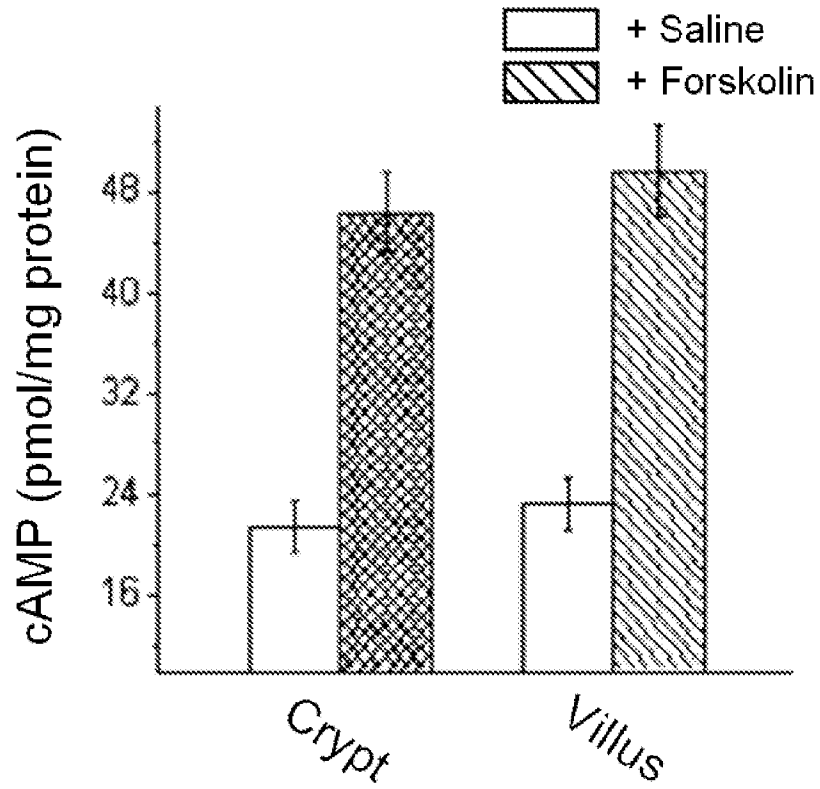
+ Saline
+ Forskolin
cAMP (pmol/mg protein)
48
40
32
24
16
Crypt
Villus

FIG. 3A

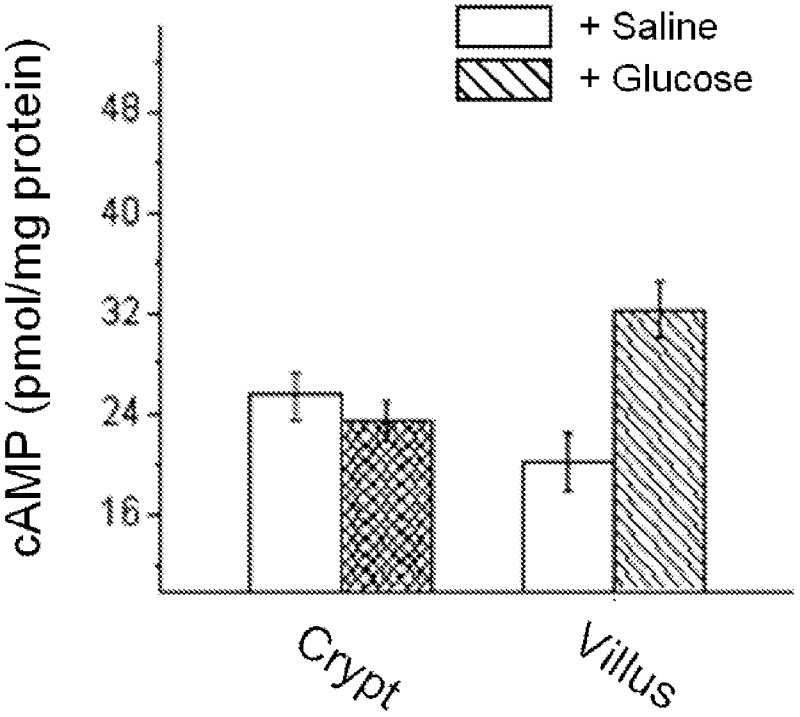
+ Saline
+ Glucose
cAMP (pmol/mg protein)
48
40
32
24
16
Crypt
Villus

FIG. 3B

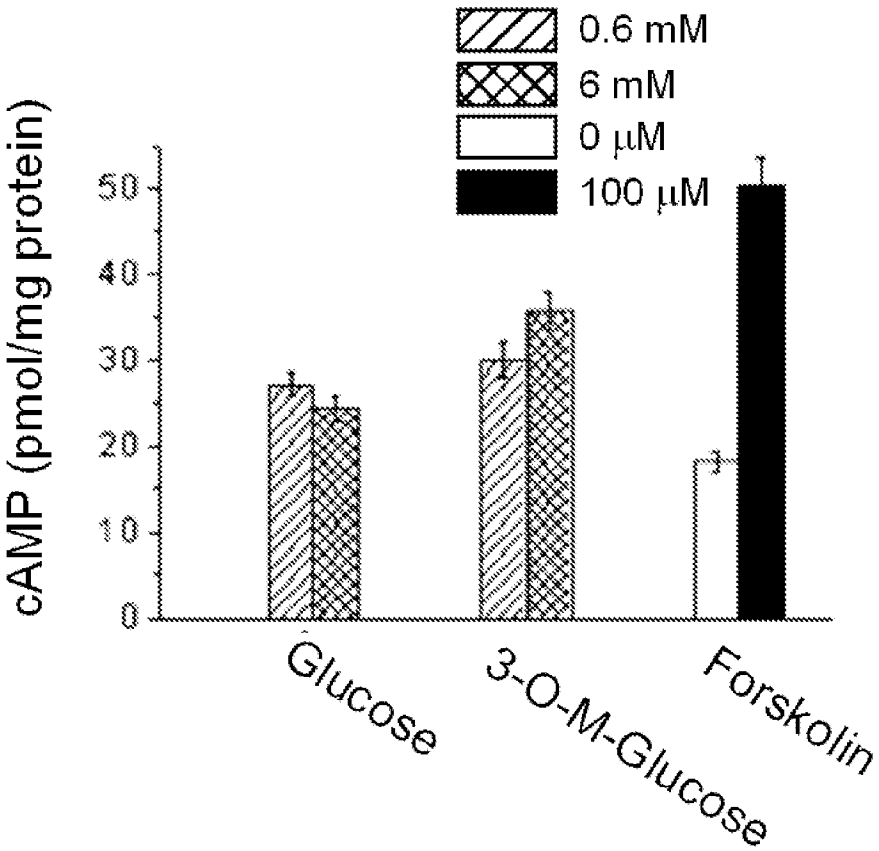
0.6 mM
6 mM
0 μM
100 μM
cAMP (pmol/mg protein)
50
40
30
20
10
0
Glucose
3-O-M-Glucose
Forskolin

FIG. 3C

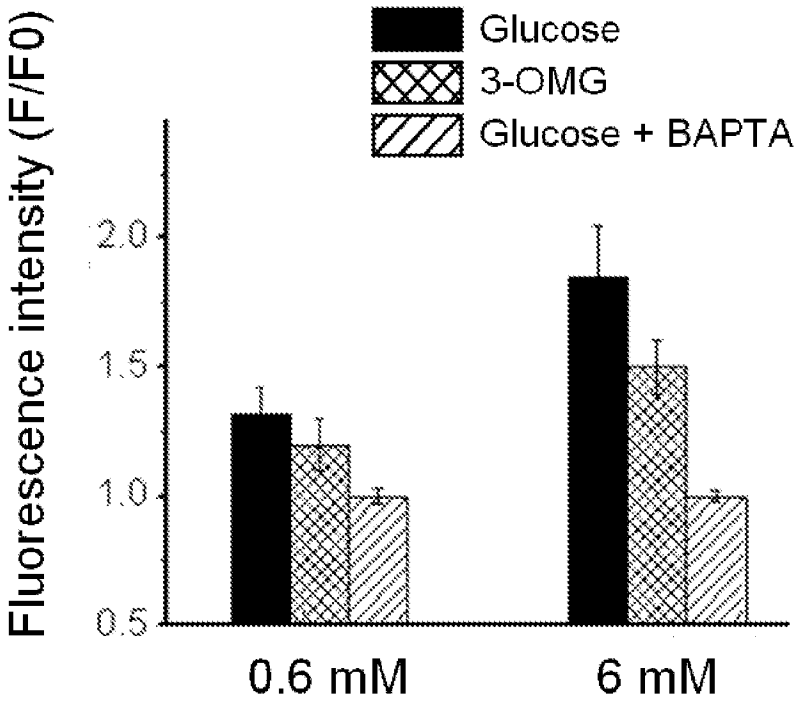
Glucose
3-OMG
Glucose + BAPTA
Fluorescence intensity (F/F0)
2.0
1.5
1.0
0.5
0.6 mM
6 mM

FIG. 4A

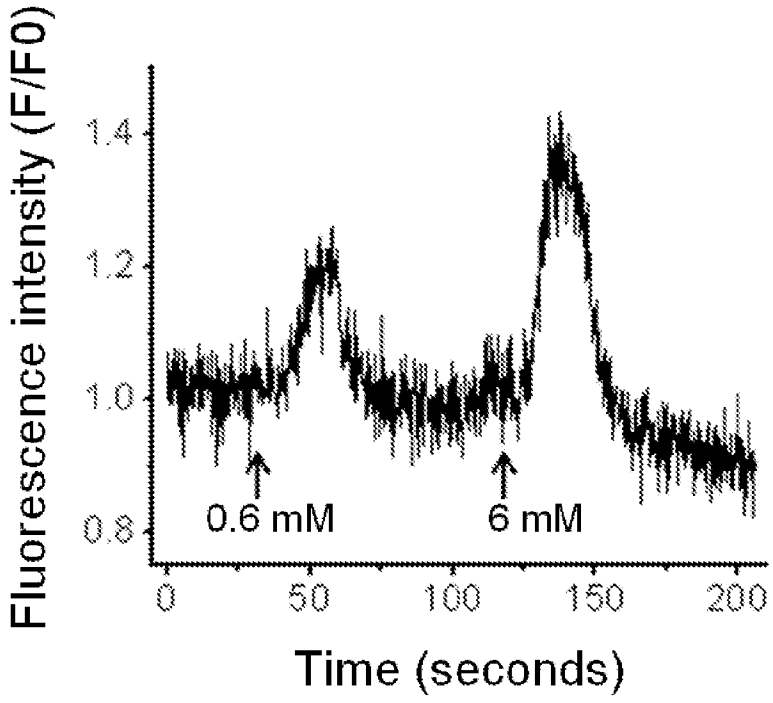
Fluorescence intensity (F/F0)
1.4
1.2
1.0
0.8
0.6 mM
6 mM
0
50
100
150
200
Time (seconds)

FIG. 4B

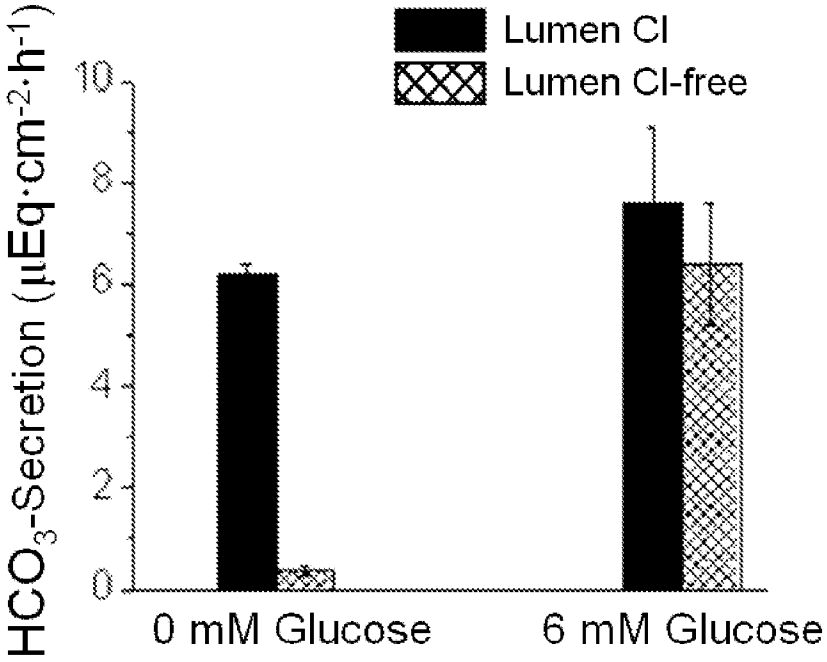
Lumen Cl
Lumen Cl-free
HCO_3-Secretion ($\mu Eq \cdot cm^{-2} \cdot h^{-1}$)
0
2
4
6
8
10
0 mM Glucose
6 mM Glucose

FIG. 5A

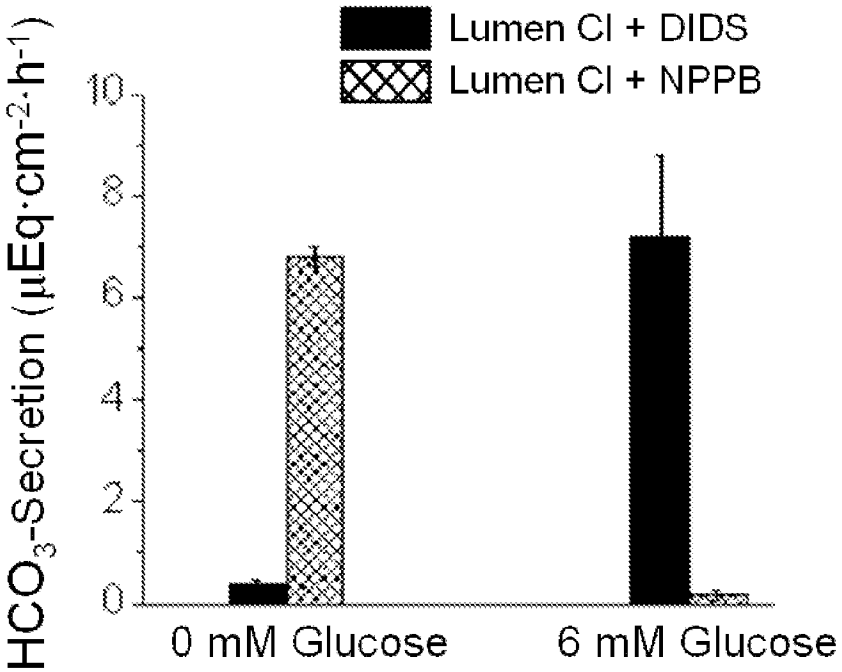
Lumen Cl + DIDS
Lumen Cl + NPPB
HCO_3-Secretion ($\mu Eq \cdot cm^{-2} \cdot h^{-1}$)
0
2
4
6
8
10
0 mM Glucose
6 mM Glucose

FIG. 5B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/025294

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/70(2006.01)i, A61K 31/505(2006.01)i, A61K 9/16(2006.01)i, A61K 9/14(2006.01)i, A61P 1/12(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 31/70; A61P 1/12; A61K 31/7016; A61K 31/47; A61K 35/78; A23L 1/09; A61K 39/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: glucose-free, oral rehydration solution, diarrhea, electrolyte

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GUTIERREZ, C. et al., 'Does an L-glutamine-containing, glucose-free, oral rehydration solution reduce stool output and time to rehydrate in children with acute diarrhoea? A double-blind randomized clinical trial', Journal of Health, Population and Nutrition, 2007, Vol. 25, No. 3, pages 278-284. See abstract; pages 279 and 282-283.	1-5,18-20
X	KR 10-2006-0130583 A (STOKELY-VAN CAMP, INC.) 19 December 2006 See abstract; and claims 1 and 12-14.	1,5,18-20
X	US 2003-0143293 A1 (SHUSHUNOV, S.) 31 July 2003 See abstract; and claims 1 and 12-13.	1,5,18-20
X	KR 10-2007-0103506 A (GLAXOSMITHKLINE BIOLOGICALS, S. A.) 23 October 2007 See abstract; and claims 1, 5, 35 and 45.	1,5,18-20
A	MATHEW, J. L., 'Non-glucose oral rehydration solution - Does it make a good thing better?', Indian Pediatrics, 2009, Vol. 46, pages 501-505. See pages 501-503.	1-5,18-20

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 May 2013 (31.05.2013)	**02 June 2013 (02.06.2013)**

Name and mailing address of the ISA/KR
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701, Republic of Korea
Facsimile No. 82-42-472-7140

Authorized officer
CHOI, Sung Hee
Telephone No. 82-42-481-8740

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/025294

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 6-17
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 6-17 pertain to methods for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2013/025294

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
KR 10-2006-0130583 A	19.12.2006	AU 2004-289359 A1	26.05.2005
		AU 2004-289359 B2	12.06.2008
		CA 2545606 A1	26.05.2005
		EP 1689254 A2	16.08.2006
		JP 2007-510758 A	26.04.2007
		US 2005-0100637 A1	12.05.2005
		US 2009-0148566 A1	11.06.2009
		US 7993690 B2	09.08.2011
		WO 2005-046360 A2	26.05.2005
		WO 2005-046360 A3	14.07.2005
US 2003-0143293 A1	31.07.2003	AU 2003-212894 A1	02.09.2003
		WO 03-063795 A2	07.08.2003
		WO 03-063795 A3	12.08.2004
KR 10-2007-0103506 A	23.10.2007	AU 2006-215761 A1	24.08.2006
		AU 2006-215761 B2	17.03.2011
		CA 2598290 A1	24.08.2006
		EP 1863526 A1	12.12.2007
		EP 1863526 B1	13.07.2011
		EP 2322211 A1	18.05.2011
		JP 2008-530165 A	07.08.2008
		JP 5118977 B2	16.01.2013
		US 2008-0166372 A1	10.07.2008
		US 2012-0237547 A1	20.09.2012
		US 8192747 B2	05.06.2012
		WO 2006-087205 A1	24.08.2006