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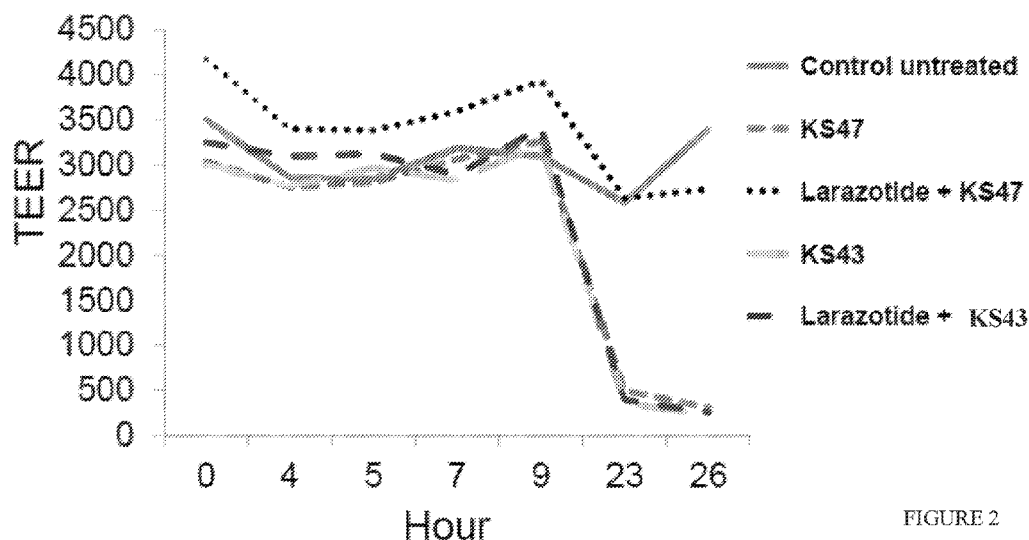


FIGURE 2

(57) Abstract: The present invention provides compositions and methods for treating or preventing environmental enteropathy and associated intestinal damage. The compositions comprise Larazotide or Larazotide derivatives.

COMPOSITIONS AND METHODS FOR TREATING OR PREVENTING ENVIRONMENTAL ENTEROPATHY

PRIORITY

This application claims priority to, and the benefit of, US Provisional
5 Application No. 62/457,279.

FIELD OF THE INVENTION

The present invention provides compositions and methods for treating diseases
and disorders associated with intestinal barrier dysfunction. Particularly, the present
invention provides methods for treating subjects having or at risk of environmental
10 enteropathy.

BACKGROUND

Environmental enteropathy, also known as tropical enteropathy or
environmental enteric dysfunction, is an intestinal disorder that occurs among subjects
who live in environments with poor sanitation and hygiene. In these subjects, chronic
15 exposure to fecal pathogens is thought to cause inflammation and structural changes in
the small intestines such as villous blunting. In children, environmental enteropathy is
implicated as a cause of malnutrition, stunted growth, impaired cognitive development,
and oral vaccine failure.

There is a need for the development of safe and effective treatments for
20 environmental enteropathy and similar conditions, including for repairing associated
intestinal damage, as well preventing the condition or associated intestinal damage in at
risk populations.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods for treating or
25 preventing environmental enteropathy (EE), including intestinal damage resulting from
EE, or in some embodiments, preventing EE or intestinal damage associated with EE.
In other aspects, the invention provides methods of preventing or treating intestinal
damage resulting from exposure to fecal pathogens. The methods comprise

administering a pharmaceutical composition comprising an effective amount of Larazotide or a derivative thereof to a subject in need. It is contemplated that administration of Larazotide restores or helps maintain intestinal barrier function, or prevents disruption of intestinal barrier function by relevant pathogens, in the treated
5 subjects. In some embodiments, Larazotide or a derivative thereof helps to prevent or reverse the dysbiosis associated with EE.

In various embodiments, the method provides for the targeted delivery of Larazotide or a Larazotide derivative to subjects afflicted with EE or at risk of EE. In some embodiments, the invention provides for administering a pharmaceutical
10 composition that releases Larazotide or a derivative thereof in the GI, such as the small intestine and/or large intestine, to prevent and/or reverse damage to the intestinal epithelium.

The compositions are safe and effective for prolonged use, and may act in concert with other agents such as antimicrobials, probiotics, and/or anti-diarrheal
15 agents.

Other aspects and embodiments of the invention will be apparent from the following detailed description.

DESCRIPTION OF THE FIGURES

FIGURE 1 shows that Larazotide does not protect T84 monolayers from
20 disruption by *Shigella*.

FIGURE 2 shows that Larazotide protects T84 cell monolayers from disruption by *Streptococcus KS47*, a bacterial species associated with damage to colonic epithelial cells as well as poor growth in Kenyan children.

FIGURE 3 shows that Larazotide's effect of protecting T84 monolayers from
25 disruption by *Streptococcus KS47* is concentration dependent.

DETAILED DESCRIPTION

The present invention provides methods for treating or preventing environmental enteropathy, comprising administering an effective amount Larazotide

or a derivative thereof to a subject or a patient in need thereof. Environmental enteropathy (EE), also known as tropical enteropathy or environmental enteric dysfunction, is an intestinal disorder that often occurs among subjects who live in environments with poor sanitation and hygiene. In these subjects, chronic exposure to fecal pathogens (e.g., in contaminated food or water) is thought to cause inflammation and structural changes in the small intestine such as villous blunting. In children, environmental enteropathy is implicated as a cause of malnutrition, stunted growth, impaired cognitive development, and oral vaccine failure. In various embodiments, methods of the invention reduce, ameliorate, or eliminate one or more symptoms of environmental enteropathy, including small intestinal and/or large intestinal epithelial damage, as well as bacterial overgrowth and dysbiosis.

The invention involves treatment with Larazotide or a derivative thereof. Larazotide is a peptide agent that promotes epithelial tight junction integrity in the gastrointestinal tract (GI). Larazotide has the amino acid sequence: Gly Gly Val Leu Val Gln Pro Gly (SEQ ID NO:1), and can be formulated for systemic or targeted release in affected portions of the GI (e.g., small intestine and/or large intestine). In some embodiments, Larazotide or derivative is targeted for release in the small and large intestines. While damage to the small intestine must be repaired to restore normal absorption of nutrients, elimination and/or amelioration of bacterial overgrowth or dysbiosis of the small intestine and/or colon may also be important to disrupt the cycle of infection, inflammation and further tissue damage.

The intestinal epithelium is the layer of cells that forms the luminal surface of the small and large intestines of the gastrointestinal (GI) tract, and represents the largest interface (more than 400 m²) between the external environment and the internal milieu. The intestinal epithelium has two important functions: absorbing nutrients and providing a barrier against harmful environmental substances such as bacteria, viruses, toxins, and food allergens.

The barrier properties of the intestinal epithelium are regulated by specialized plasma membrane structures known as tight junctions. Alterations in tight junctions can result in disruptions of the intestinal barrier functions and increased intestinal permeability. An intact intestinal barrier prevents the permeation of antigens,

endotoxins, proinflammatory substances into the body, whereas intestinal disintegrity allows their entry, which may trigger or exacerbate local or systemic inflammatory disease. In addition, as disclosed herein, Larazotide can prevent barrier disruption by some pathogens associated with EE.

- 5 Animal models for EE are available, including as disclosed by Brown, EM et al. Diet and specific microbial exposure trigger features of environmental enteropathy in a novel murine model (2015) *Nat. Commun.* 6:7806.

 In various embodiments, the subject having or at risk of EE is a pediatric human subject. In various embodiments, the pediatric subject may be from about 1 month to
10 about 18 years old. For example, the subject may be from 1 month to 12 months old, from about 12 months to about 36 months old. In some embodiments, the subject is between 1 and 18 years old, such as from about 1 to about 10 years old, or from about 5 to about 10 years old, or from about 10 to about 15 years old. In some embodiments, the pediatric subject is an infant (e.g., less than about 9 months old). In still other
15 embodiments, the subject is an adult human subject.

 In some embodiments, the invention provides for administration of Larazotide or a derivative thereof to a subject living in environments of poor sanitation (e.g., including but not limited to developing countries). In some embodiments, the invention provides for administration of the Larazotide or derivative to subjects exposed to fecal
20 pathogens, such as in contaminated food or water. Fecal pathogens can disrupt gut intestinal epithelium, causing chronic infection associated with EE.

 In various embodiments, the methods of the invention comprise treating a subject with Larazotide or derivative thereof. Larazotide is a peptide agent that promotes tight junction integrity in the gastrointestinal tract (GI). Larazotide comprises
25 the amino acid sequence: Gly Gly Val Leu Val Gln Pro Gly (SEQ ID NO:1), and can be formulated for systemic or targeted release in affected portions of the GI (e.g., small intestine and/or large intestine).

 In some embodiments, the active agent is a Larazotide derivative, for example, having one or more amino acid modifications, such as substitutions, deletions, and/or
30 insertions. For example, the derivative may have 1, 2, 3, or 4 amino acid modifications

independently selected from deletions, insertions, and/or substitutions with respect to SEQ ID NO:1. Larazotide derivatives are described in US 8,785,374, US 8,957,032, and US 9,279,807, which are hereby incorporated by reference in their entirety. In some embodiments, the derivative has one or more non-genetically encoded amino acids, or one or more (or all) D-amino acids. The term "Larazotide" or "Larazotide treatment" refers to treatment with Larazotide or a derivative that promotes tight junction integrity.

Larazotide or derivative may be administered in any suitable form, including as a salt. For example, Larazotide may be administered as an acetate salt. Salts of Larazotide, including the acetate salt and hydrochloride salt, are described in US 2013/0281384, which is hereby incorporated by reference in its entirety. Alternative salts may be employed, including any pharmaceutically acceptable salt of the peptide such as those listed in Journal of Pharmaceutical Science, 66, 2-19 (1977) and The Handbook of Pharmaceutical Salts; Properties, Selection, and Use. P. H. Stahl and C. G. Wermuth (eds.), Verlag, Zurich (Switzerland) 2002, which are hereby incorporated by reference in their entirety.

In various embodiments, the pharmaceutical compositions comprising Larazotide or Larazotide derivatives can take the form of tablets, pills, pellets, capsules, capsules containing liquids, capsules containing multiparticulates, powders, solutions, emulsion, drops, suppositories, aerosols, sprays, suspensions, delayed-release formulations, sustained-release formulations, or controlled-release formulations. In some embodiments, the pharmaceutical compositions are formulated for oral administration, and may include pharmaceutically acceptable excipients or carriers.

Various methods may be used to formulate and/or deliver the Larazotide or derivative to a location of interest. In some embodiments, the Larazotide is formulated for targeted delivery to the gastrointestinal tract including the stomach, small intestine, large intestine and/or rectum including all subsections thereof. By targeting release of Larazotide or derivative in the affected region(s) (e.g. duodenum, jejunum and ileum, colon transversum, colon descendens, colon ascendens, colon sigmoidenum and cecum), tight junction integrity at any portion of the GI can be improved, and in some embodiments dysbiosis ameliorated.

In some embodiments, the composition is formulated to release in the small intestine, including one or more of the duodenum, jejunum, and/or the ileum.

In these or other embodiments, the composition is formulated to release in the large intestine, including one or more of the cecum, the ascending colon, the transverse colon, the descending colon, and/or the sigmoid colon.

In various embodiments, the composition may be formulated to have sustained-release profiles, *i.e.* slow release of the Larazotide in the GI tract over an extended period of time. In various embodiments, the composition may be formulated to have a delayed-release profile, *i.e.* not immediately release the Larazotide upon ingestion; rather, postponement of the release until the composition is lower in the gastrointestinal tract; for example, for release in the small intestine (*e.g.*, one or more of duodenum, jejunum, ileum) and/or the large intestine (*e.g.*, one or more of cecum, ascending, transverse, descending or sigmoid portions of the colon, and rectum). In an embodiment, the pharmaceutical composition is formulated to have a delayed-release profile as described in, for example, U.S. Patent No. 8,168,594, the entire contents of which are hereby incorporated by reference.

For example, the Larazotide or derivative may be administered to the duodenum of the patient, as an oral dosage, delayed-release composition that contains Larazotide-coated beads that are stable in gastric fluid and unstable in intestinal fluid so as to substantially release the peptide in the duodenum. The composition may further comprise a second population of beads with a pH-dependent coating to affect release of the peptide in the jejunum of the patient. For example, the second population of beads may release the Larazotide or derivative about 30 minutes after the beads releasing peptide in the duodenum. The oral dosage composition can be in the form of a capsule or tablet. The pH-dependent coating in some embodiments is a 1:1 co-polymer of methacrylic acid and ethyl acrylate, wherein the thickness of the layer determines the release profile of each bead. The beads may have one or more additional coatings such as a base coat, a separating layer, and an overcoat layer.

In an exemplary oral dosage composition, an effective amount of Larazotide or derivative (*e.g.*, as the acetate salt) is provided in first delayed-release particles that are capable of releasing Larazotide or derivative in the duodenum of a patient, and second

delayed release particles that are capable of releasing Larazotide or derivative in the jejunum of a patient. Each particle has a core particle, a coat comprising Larazotide or derivative over the core particle, and a delayed-release coating (e.g., a 1:1 co-polymer of acrylate and methacrylate) outside the coat comprising Larazotide or derivative.

- 5 Whereas the first delayed-release particles release at least 70% of the Larazotide or derivative in the first delayed-release particles by about 60 minutes of exposure to simulated intestinal fluid having a pH of greater than 5; the second delayed-release particles release at least 70% of the Larazotide or derivative by about 30 and about 90 minutes of exposure to simulated intestinal fluid having a pH of greater than 5.

- 10 In another example, the Larazotide or derivative may be administered to the colon of a patient, as an oral dosage, modified-release composition. Various colon-specific delivery approaches may be utilized. For example, the modified release formulation may be formulated using a colon-specific drug delivery system (CODES) as described for example, in Li *et al.*, AAPS PharmSciTech (2002), 3(4): 1-9, the entire
15 contents of which are incorporated herein by reference. Drug release in such a system is triggered by colonic microflora coupled with pH-sensitive polymer coatings. For example, the formulation may be designed as a core tablet with three layers of polymer. The first coating is an acid-soluble polymer (e.g., EUDRAGIT E), the outer coating is enteric, along with a hydroxypropyl methylcellulose barrier layer interposed in
20 between. In another embodiment, colon delivery may be achieved by formulating the Larazotide with specific polymers that degrade in the colon such as, for example, pectin. The pectin may be further gelled or crosslinked with a cation such as a zinc cation. Additional colon specific formulations include, but are not limited to, pressure-controlled drug delivery systems (prepared with, for example, ethylcellulose) and
25 osmotic controlled drug delivery systems (*i.e.*, ORDS-CT).

- In various embodiments, the compositions of the present invention may use one or more modified-release coatings such as delayed-release coatings to provide for effective, delayed yet substantial delivery of the Larazotide or derivative to the GI tract. For example, a composition can be enteric coated to delay release of the Larazotide or
30 derivative until it reaches the small intestine or the large intestine.

In an embodiment, the composition may remain essentially intact, or may be essentially insoluble, in gastric fluid. In some embodiments, the stability of the delayed-release coating can be pH dependent. Delayed-release coatings that are pH dependent will be substantially stable in acidic environments (pH of about 5 or less),
5 and substantially unstable in near neutral to alkaline environments (pH greater than about 5). For example, the delayed-release coating may essentially disintegrate or dissolve in near neutral to alkaline environments such as are found in the small intestine (*e.g.* one or more of the duodenum, jejunum, and ileum) and/or large intestine (*e.g.* one or more of the cecum, ascending colon, transverse colon, descending colon,
10 and sigmoid colon).

In one embodiment, the delayed-release coating includes an enteric agent that is substantially stable in acidic environments and substantially unstable in near neutral to alkaline environments. In an embodiment, the delayed-release coating contains an enteric agent that is substantially stable in gastric fluid. The enteric agent can be
15 selected from, for example, solutions or dispersions of methacrylic acid copolymers, cellulose acetate phthalate, hydroxypropylmethyl cellulose phthalate, polyvinyl acetate phthalate, carboxymethylethylcellulose, and EUDRAGIT®-type polymer (poly(methacrylic acid, methylmethacrylate), hydroxypropyl methylcellulose acetate succinate, cellulose acetate trimellitate, shellac or other suitable enteric coating
20 polymers. The EUDRAGIT®-type polymer include, for example, EUDRAGIT® FS 30D, L 30 D-55, L 100-55, L 100, L 12,5, L 12,5 P, RL 30 D, RL PO, RL 100, RL 12,5, RS 30 D, RS PO, RS 100, RS 12,5, NE 30 D, NE 40 D, NM 30 D, S 100, S 12,5, and S 12,5 P. In some embodiments, one or more of EUDRAGIT® FS 30D, L 30 D-55, L 100-55, L 100, L 12,5, L 12,5 P RL 30 D, RL PO, RL 100, RL 12,5, RS 30 D, RS
25 PO, RS 100, RS 12,5, NE 30 D, NE 40 D, NM 30 D, S 100, S 12,5 and S 12,5 P is used. The enteric agent may be a combination of the foregoing solutions or dispersions.

In another embodiment, the delayed-release coating may degrade as a function of time when in aqueous solution without regard to the pH and/or presence of enzymes in the solution. Such a coating may comprise a water insoluble polymer. Its solubility
30 in aqueous solution is therefore independent of the pH. The term “pH independent” as used herein means that the water permeability of the polymer and its ability to release pharmaceutical ingredients is not a function of pH and/or is only very slightly

dependent on pH. Such coatings may be used to prepare, for example, sustained release formulations. Suitable water insoluble polymers include pharmaceutically acceptable non-toxic polymers that are substantially insoluble in aqueous media, *e.g.*, water, independent of the pH of the solution. Suitable polymers include, but are not limited to, 5 cellulose ethers, cellulose esters, or cellulose ether-esters, *i.e.*, a cellulose derivative in which some of the hydroxy groups on the cellulose skeleton are substituted with alkyl groups and some are modified with alkanoyl groups. Examples include ethyl cellulose, acetyl cellulose, nitrocellulose, and the like. Other examples of insoluble polymers include, but are not limited to, lacquer, and acrylic and/or methacrylic ester polymers, 10 polymers or copolymers of acrylate or methacrylate having a low quaternary ammonium content, or mixture thereof and the like. Other examples of insoluble polymers include EUDRAGIT RS®, EUDRAGIT RL®, EUDRAGIT NE®, polyvinyl esters, polyvinyl acetals, polyacrylic acid esters, butadiene styrene copolymers, and the like.

15 In some embodiments, the stability of the pharmaceutical composition can be enzyme-dependent. Delayed-release coatings that are enzyme dependent will be substantially stable in fluid that does not contain a particular enzyme and substantially unstable in fluid containing the enzyme. The delayed-release coating will essentially disintegrate or dissolve in fluid containing the appropriate enzyme. Enzyme-dependent 20 control can be brought about, for example, by using materials which release the active ingredient only on exposure to enzymes in the intestine. In certain embodiments, the stability of the composition can be dependent on the presence of a microbial enzyme present in the gut flora. Accordingly, in various embodiments, the delayed-release coating is degraded by a microbial enzyme present in the gut flora. In an embodiment, 25 the delayed-release coating is degraded by a bacteria present in the small intestine. In another embodiment, the delayed-release coating is degraded by a bacteria present in the large intestine.

The present invention also provides for compositions that release multiple doses of the Larazotide or derivative along the gastrointestinal tract. For example, the 30 composition and/or formulation can release multiple doses of the Larazotide or derivative at different locations along the intestines, at different times, and/or at different pH. The overall release profile of such a formulation may be adjusted using,

for example, multiple particle types or multiple layers. For example, in one embodiment, the first dose of the Larazotide or derivative may be formulated for release in, for example, the small intestine (*e.g.*, one or more of duodenum, jejunum, ileum), whereas the second dose is formulated for delayed release in, for example, the large intestine (*e.g.*, one or more of cecum, ascending, transverse, descending or sigmoid portions of the colon, and rectum). In another example, the first dose of the Larazotide or derivative may be formulated for release in, for example, the small intestine (*e.g.*, one or more of duodenum, jejunum, ileum), whereas the second dose is formulated for delayed release in, for example, another part of the small intestine (*e.g.*, one or more of duodenum, jejunum, ileum). In another embodiment, the first dose of the Larazotide or derivative may be formulated for release in, for example, the large intestine (*e.g.*, one or more of cecum, ascending, transverse, descending or sigmoid portions of the colon, and rectum), whereas the second dose is formulated for delayed release in, for example, another part of the large intestine (*e.g.*, one or more of cecum, ascending, transverse, descending or sigmoid portions of the colon, and rectum). In various embodiments, the composition and/or formulation may release at least one dose, at least two doses, at least three doses, at least four doses, or at least five doses of the Larazotide or derivative at different locations along the intestines, at different times, and/or at different pH.

Larazotide and derivatives can be administered in unit dosage forms (*e.g.*, tablets, capsules, or solutions). For example, Larazotide (or derivative thereof) can be administered in compositions that release Larazotide in the small intestine at a dose of from about 0.1 mg to about 5 mg, or at from about 0.1 mg to about 2 mg, or at from about 0.25 mg to about 2 mg, or at from about 0.25 mg to about 1 mg, or from about 0.25 to about 0.5 mg. In some embodiments, Larazotide or derivative is released in at least two locations of the small intestine selected from the duodenum, jejunum, and ileum. In some embodiments, Larazotide or derivative is released in each of the duodenum, jejunum, and ileum.

Additionally or alternatively, Larazotide or derivative is administered in compositions that release in the large intestine at a dose of from about 0.1 mg to about 5 mg, or at from about 0.1 mg to about 2 mg, or at from about 0.25 mg to about 2 mg, or at from about 0.25 mg to about 1 mg, or from about 0.25 to about 0.5 mg. In some

embodiments, Larazotide or derivative is released in at least two or three locations of the large intestine selected from the cecum, ascending colon, transverse colon, descending colon, and sigmoid colon.

Compositions targeting the small intestine and large intestine can be delivered
5 in the same or different composition.

In accordance with certain embodiments of the invention, Larazotide or derivative is administered more than once daily. For example, Larazotide or derivative may be administered about two times daily, about three times daily, about four times daily, or about five times daily.

10 In various embodiments, the Larazotide (or derivative) regimen may be administered for a prolonged period. For example, the Larazotide (or derivative) regimen may be administered for at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 4 weeks, at least about 8 weeks, or at least about 12 weeks. In some embodiments, the Larazotide (or derivative) regimen is administered
15 for at least about 1 month, at least about 2 months, at least about 3 months, at least about 4 months, at least about 5 months, or at least about 6 months. In some embodiments, the Larazotide (or derivative) regimen is administered for at least about 1 year or more.

In various embodiments, Larazotide or derivative is administered with one or
20 more agents that help to ameliorate dysbiosis and/or normalize the gut microbiome.

In some embodiments, Larazotide or derivative is administered with antibiotic therapy. Antibiotics suitable for use in the present invention include, but are not limited to, cephalosporin antibiotics (cephalexin, cefuroxime, cefadroxil, cefazolin, cephalothin, cefaclor, cefamandole, cefoxitin, cefprozil, and ceftobiprole);
25 fluoroquinolone antibiotics (cipro, Levaquin, floxin, tequin, avelox, and norflox); tetracycline antibiotics (tetracycline, minocycline, oxytetracycline, and doxycycline); penicillin antibiotics (amoxicillin, ampicillin, penicillin V, dicloxacillin, carbenicillin, vancomycin, and methicillin); monobactam antibiotics (aztreonam); and carbapenem antibiotics (ertapenem, doripenem, imipenem/cilastatin, and meropenem). In some
30 embodiments, the Larazotide (or derivative) regimen is administered after antibiotic

treatment, to prevent reestablishment of infection by environmental pathogens. In some embodiments, administration of Larazotide helps to establish a healthy microbiome after antibiotic therapy.

In some embodiments, Larazotide is administered with an antiviral agent. Exemplary antiviral agents include, but are not limited to, Abacavir, Acyclovir, Adefovir, Amprenavir, Atazanavir, Cidofovir, Darunavir, Delavirdine, Didanosine, Docosanol, Efavirenz, Elvitegravir, Emtricitabine, Enfuvirtide, Etravirine, Famciclovir, and Foscarnet. In some embodiments, the Larazotide (or derivative) regimen is administered after antiviral treatment, to promote integrity of the gut epithelium.

In some embodiments, Larazotide or derivative is administered with a probiotic. Probiotics suitable for use in the present invention include, but are not limited to, *Saccharomyces boulardii*; *Lactobacillus rhamnosus* GG; *Lactobacillus plantarum* 299v; *Clostridium butyricum* M588; *Clostridium difficile* VP20621 (non-toxigenic *C. difficile* strain); combination of *Lactobacillus casei*, *Lactobacillus acidophilus* (Bio-K + CL1285); combination of *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* (Actimel); combination of *Lactobacillus acidophilus*, *Bifidobacterium bifidum* (Florajen3); combination of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus delbrueckii* subsp. *bulgaricus*, *Lactobacillus bulgaricus casei*, *Lactobacillus bulgaricus plantarum*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Bifidobacterium breve*, and *Streptococcus salivarius* subsp. *thermophilus* (VSL#3)). Without wishing to be bound by theory, it is believed that administering Larazotide with probiotic can more effectively overcome or prevent dysbiosis, particularly in subjects susceptible to EE. For example, it is believed that Larazotide can prevent damage to the intestinal epithelium by some microorganisms associated with EE. In some embodiments, by promoting normal gut epithelium, probiotics can be more effective. In some embodiments, Larazotide and probiotic are administered after antibiotic therapy.

In some embodiments, the Larazotide is administered during or after an antidiarrheal agent that resolves acute or chronic symptoms. Antidiarrheal agents suitable for use in the present invention include, but are not limited to, DPP-IV inhibitors, natural opioids, such as tincture of opium, paregoric, and codeine, synthetic

opioids, such as diphenoxylate, difenoxin and loperamide, bismuth subsalicylate, lanreotide, vapreotide and octreotide, motilin antagonists, COX2 inhibitors like celecoxib, glutamine, thalidomide and traditional antidiarrheal remedies, such as kaolin, pectin, berberine and muscarinic agents.

5

EXAMPLES

Diarrhea and enteric enteropathy (EE) are inflammatory diseases. EE is a chronic disease involving a vicious cycle of inflammation and nitric oxide release and utilization resulting in poor growth.

Bacterial 16S-defined taxa were evaluated using Metagenomeseq (99% similar sequences, Operational Taxonomic Unit (OTU)) for association with poor growth. Bottom and top quartile from 1735 control children from GEMS were evaluated.

A number of OTUs were associated with poor growth (Weight Adjusted Z-score, WAZ), and which vary by age (Table 1). Many OTUs of *Streptococcus lutetiensis* and *E. coli* were associated with poor growth in all five age categories (*S. lutetiensis* and *E. coli* species were named at 97% similarity, and thus many OTUs clustered at 99% receive the same name. Individual OTUs may not truly be a member of the nominal species).

Table 1: Number of OTU's associated with poor growth by age in months

	0-5 mo	6-11 mo.	12-17 mo.	18-23 mo.	>24 mo.
<i>Streptococcus lutetiensis</i>	5	37	7	1	2
<i>Streptococcus salivarius</i>	1	4	4	1	3
<i>Prevotella copri</i>	1		18	3	10
<i>Prevotella</i> sp. DJF_RP53		1	18	1	6

<i>Prevotella sp.</i> BI-42			10		1
<i>Escherichia coli</i>	8	19	4	14	3
<i>Shigella sonnei</i>	4	12	4	1	
<i>Faecalibacterium prausnitzii</i>		2	3	2	80

Available data are consistent with the hypothesis that *Streptococcus spp.* and *E. coli* are associated with poor growth. *Streptococcus KS47* is associated with poor growth in Kenyan children as well as damage to the colonic epithelial monolayer.

The impact of *Streptococcus KS47* and other bacterial species associated with diarrhea or chronic colonic infection on T84 cells was examined, along with the potential role of Larazotide to prevent damage. T84 cells are a human colonic epithelial cell line that differentiates and creates tight junctions. T84 cells are a model for colonic inflammation.

As shown in Figure 1, Larazotide does not protect T84 monolayers from disruption by *Shigella*. However, Larazotide does protect T84 cells from disruption by certain *Streptococcus sp.* Both *Streptococcus KS47* and *Streptococcus KS43* disrupt T84 monolayers, but Larazotide prevents damage from *Streptococcus KS47* (Figure 2). This effect of Larazotide is concentration dependent (Figure 3).

CLAIMS

1. A method for treating or preventing environmental enteropathy (EE) or intestinal damage resulting from exposure to fecal pathogens, comprising administering an effective amount of a composition comprising Larazotide or a derivative thereof to a
5 subject in need thereof.
2. The method of claim 1, wherein the subject is administered a composition releasing Larazotide or a derivative in the small and/or large intestine.
3. The method of claim 2, wherein the composition releases Larazotide or derivative in one or more of the duodenum, jejunum, and/or the ileum.
- 10 4. The method of claim 2, wherein the composition releases Larazotide or derivative in the cecum, the ascending colon, the transverse colon, the descending colon, and/or the sigmoid colon.
5. The method of claim 2, wherein the subject is administered one or more compositions releasing Larazotide in the small and large intestine.
- 15 6. The method of any one of claims 1 to 5, wherein the subject is a pediatric subject.
7. The method of any one of claims 1 to 5, wherein the subject is an adult.
8. The method of any one of claims 1 to 7, wherein the subject has EE.
9. The method of any one of claims 1 to 7, wherein the subject is at risk of EE.
- 20 10. The method of claim 9, wherein the subject lives in an environment of poor sanitation or prevalence of EE.
11. The method of claim 9 or 10, wherein the subject is exposed to food or water contaminated with fecal pathogens.
12. The method of any one of claims 1 to 11, wherein the composition is a
25 controlled-release or sustained-release formulation.

13. The method of claim 12, wherein the composition releases Larazotide or derivative in the small intestine at a dose of from about 0.1 mg to about 5 mg.
14. The method of claim 13, wherein the composition releases Larazotide or derivative in the small intestine at a dose of from about 0.1 mg to about 1 mg.
- 5 15. The method of claim 12, wherein the composition releases Larazotide or derivative in the large intestine at a dose of from about 0.1 mg to about 5 mg.
16. The method of claim 15, wherein the composition releases Larazotide or derivative in the large intestine at a dose of from about 0.1 mg to about 1 mg.
17. The method of any one of claims 12 to 16, wherein a single composition targets
10 release of Larazotide or derivative in the small intestine and large intestine.
18. The method of any one of claims 12 to 16, wherein different compositions target release of Larazotide or derivative in the small intestine and large intestine.
19. The method of any one of claims 1 to 18, wherein the composition is administered more than once daily.
- 15 20. The method of any one of claims 1 to 19, the Larazotide or derivative regimen is for a prolonged period.
21. The method of any one of claims 1 to 20, wherein the subject further receives antibiotic therapy.
22. The method of claim 21, wherein antibiotic therapy is delivered before or
20 during therapy with Larazotide or derivative.
23. The method of claim 21, wherein antiviral therapy is delivered before or during therapy with Larazotide or derivative.
24. The method of any one of claims 1 to 22, wherein the subject further receives a probiotic.
- 25 25. The method of claim 24, wherein the probiotic is delivered after antibiotic therapy, together with therapy with Larazotide or derivative.

26. The method of any one of claims 1 to 23, wherein the Larazotide or derivative is administered with or after an antidiarrheal agent.

FIGURE 1

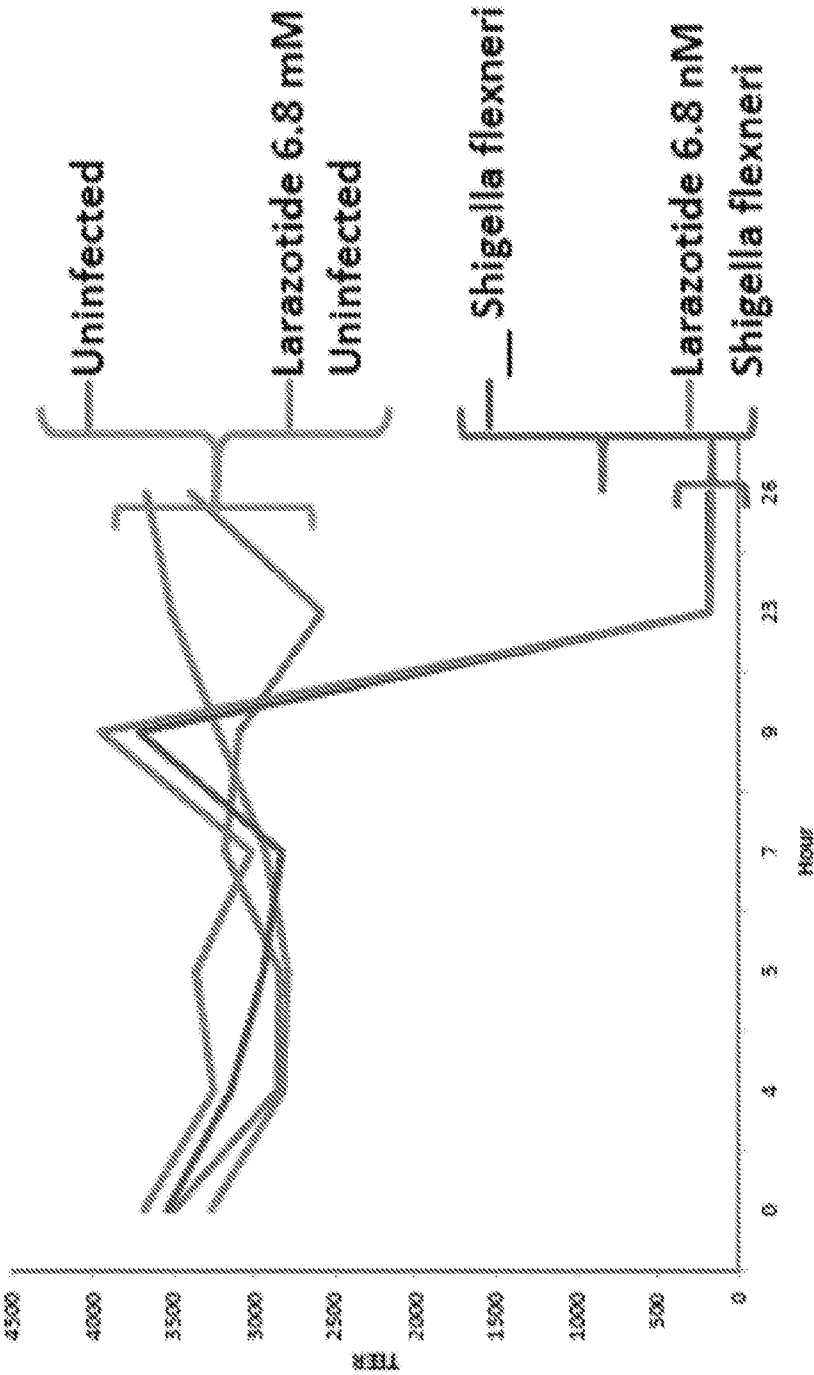


FIGURE 2

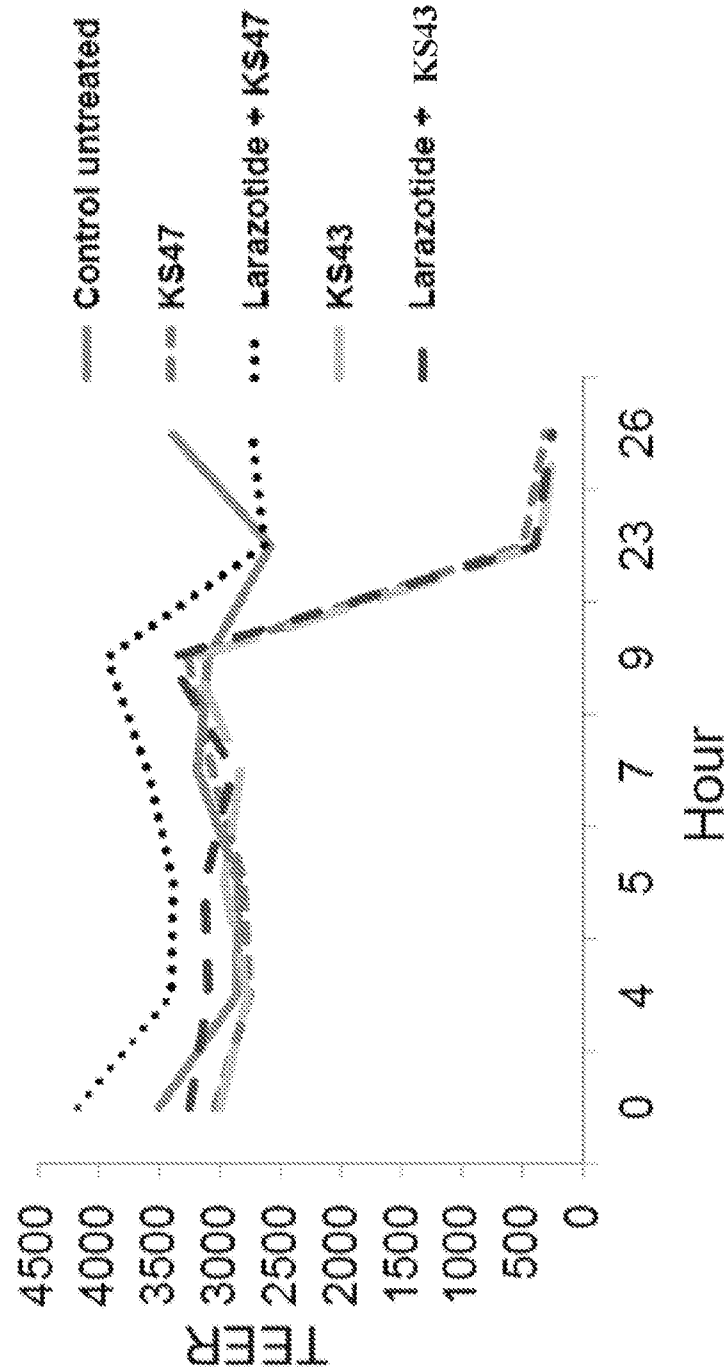
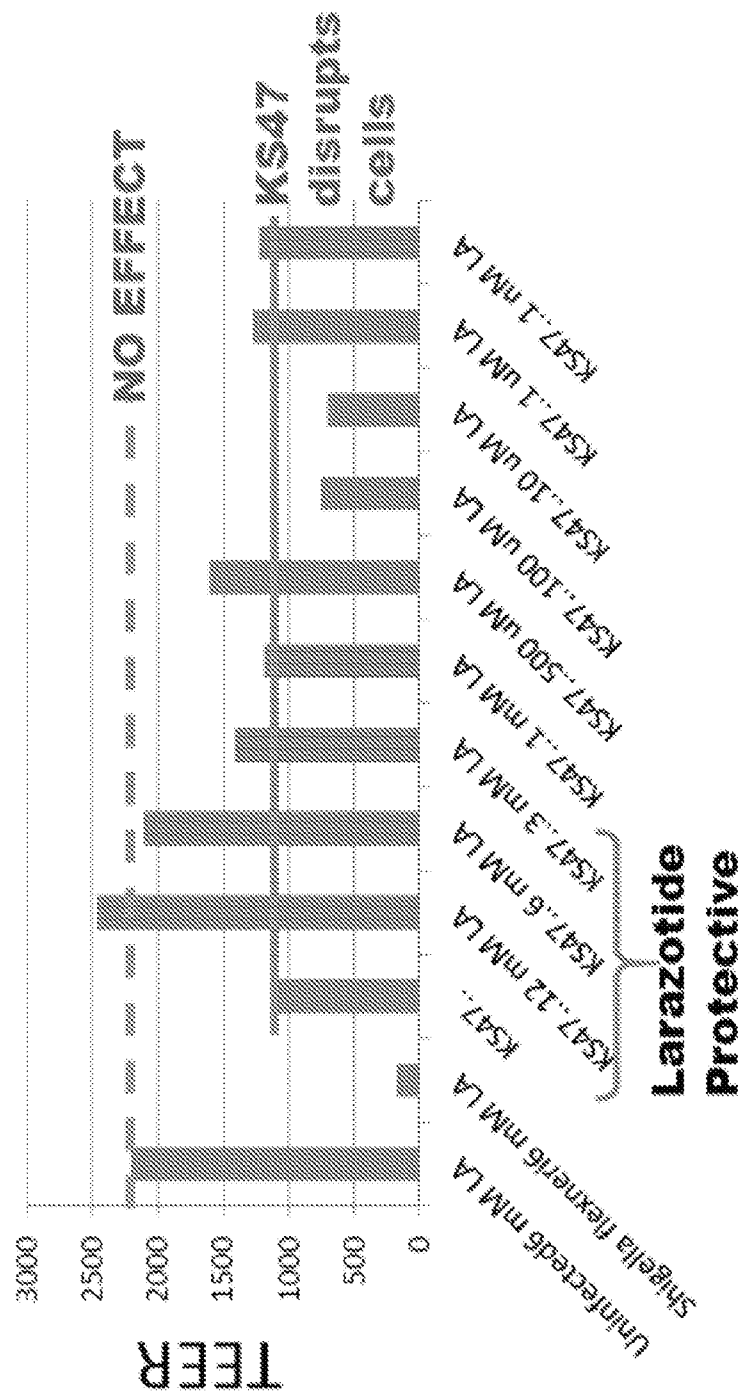


FIGURE 3



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/17811

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 38/08; A61P 1/00; C07C 53/10; C07K 7/04, 7/06 (2018.01)

CPC - A61K 38/08; C07C 53/10; C07K 7/04, 7/06

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)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	(PETRI, WA et al.) Environmental Enteropathy and Malnutrition: Do We Know Enough to Intervene. BMC Medicine 2014, vol. 12, pages 187 (pages 1-5); table 1; page 1, left column, first paragraph; page 3, right column, second paragraph; page 4, left column, third paragraph	1-5, 6/1-5, 7/1-5
Y	US 2016/0022760 A1 (PERROW, W et al.) 28 January 2016; paragraphs [0026], [0031], [0066]-[0069]	1-5; 6/1-5, 7/1-5

☐ 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 another 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

20 March 2018 (20.03.2018)

Date of mailing of the international search report

09 APR 2018

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

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Authorized officer

Shane Thomas

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/17811

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.:
because they relate to subject matter not required to be searched by this Authority, namely:

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.: 8-26
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 additional 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 additional fees, this Authority did not invite payment of additional fees.
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.