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(54) Title: METHODS AND COMPOSITIONS OF TREATING AND PREVENTING INTESTINAL INJURY AND DISEASES RELATED TO TIGHT JUNCTION DYSFUNCTION

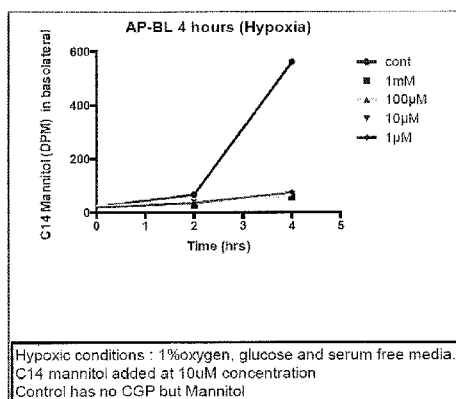


Fig. 4. Increase in mannitol penetration in Caco-2 cells 4 hours after the administration of calcium glycerophosphate (CGP) to the cells.

(57) **Abstract:** Methods and compositions for treating and preventing intestinal injury are described. In particular, methods and delayed-release enteric coated compositions of glycerophosphate salts for treating and preventing an intestinal injury, such as exercise-induced intestinal injury, intestinal injury induced or aggravated by ingestion of a nonsteroidal anti-inflammatory drug (NSAID), or exercise-induced intestinal injury aggravated by an NSAID, are described. Orally or nasally administering a therapeutically effective amount of a glycerophosphate salt, particularly calcium glycerophosphate, to a subject engaging in exercise, particularly a subject engaging in exercise and ingesting a NSAID for the treatment or prevention of musculoskeletal pain resulting from exercise, reduces exercise-induced intestinal injury, particularly exercise-induced intestinal injury aggravated by the NSAID. A method of increasing tight junction integrity in a subject in need thereof is also described. The method involves administering to the subject a composition comprising an effective amount of glycerophosphate, such as calcium glycerophosphate. A method of treating or preventing a disease related to tight junction dysfunction, such as an inflammatory disease or a cancer, is also described.



TITLE OF THE INVENTION

[0001] Methods and Compositions for Treating and Preventing Intestinal Injury and Diseases Related to Tight Junction Dysfunction

CROSS-REFERENCE TO RELATED APPLICATIONS

5 [0002] This application claims priority to U.S. Patent Application Serial No. 61/766,976, filed February 20, 2013, U.S. Patent Application Serial No. 61/827,080, filed May 24, 2013, and U.S. Patent Application Serial No. 61/897,672, filed October 30, 2013, the disclosures of which are herein incorporated by reference.

FIELD OF THE INVENTION

10 [0003] The invention relates to methods and compositions for treating and preventing intestinal injury. In particular, the invention provides a method of reducing, alleviating, or preventing intestinal injury in a subject, such as that induced by exercise or induced or aggravated by the ingestion of a nonsteroidal anti-inflammatory drug, using a glycerophosphate salt. The invention also relates to methods and compositions for regulating tight junctions and for
15 preventing or treating diseases related to tight junction dysfunction.

BACKGROUND OF THE INVENTION

[0004] Blood flow distribution in the human and animal body is a function of oxygen demand, which in turn is dependent on activity. That is, flow is differentially distributed to those vascular beds serving organs with the highest oxygen demand. This is accomplished by
20 constricting the vessels supplying low demand organs and relaxing those supplying high demand organs.

[0005] Blood flow exerts shear force on the vessel wall, stimulating the synthesis of cytoprotective agents such as nitric oxide and prostaglandins. Interruptions or reduction of flow reduces shear force, reducing the synthesis of these agents, and setting tissue up for ischemic
25 damage (i.e., damage due to reduced or restricted blood flow).

[0006] The splanchnic circulation refers to the circulation of the blood to organs of the gastrointestinal tract, including the stomach, small intestine, colon, pancreas, liver, and spleen. The splanchnic flow (blood flow to the gut) is highly variable, and when blood flow to the gut is reduced, the gut may be operating under conditions of mild to moderate ischemia.

30 [0007] In particular, the splanchnic flow is curtailed during heavy exercise because blood flow is diverted to skeletal muscle, heart, and skin. Thus, during vigorous exercise the gut often

operates under conditions of mild to moderate ischemia. This effect is greatly enhanced by concomitant ingestion of ibuprofen, a non-steroidal anti-inflammatory agent or NSAID (see van Wijk *et al.*, *Medicine & Science in Sports & Exercise* 44, 2257-2262 (2012); hereinafter referred to as “van Wijk”).

5 [0008] NSAIDs block the synthesis of prostaglandins, substances that mediate pain and inflammation. However, prostaglandins are also cytoprotective, particularly to the gastrointestinal (GI) tract. Consequently, the chronic use of NSAIDs is associated with adverse GI effects such as ulcers and compromised intestinal barrier function and, as a result, increased gut permeability (E. Focalin, *Ann. Clin. Lab. Sci.* 2, 67-81 (1998)).

10 [0009] Athletes routinely seek ways to prevent exercise-induced physical pain, subsequently improving their physical performance. Commonly, athletes use NSAIDs before, during and/or after physical activity to treat existing musculoskeletal pain, or in anticipation of musculoskeletal pain associated with, or induced by, exercise and physical activity. Ingestion of NSAIDs alone can cause intestinal injury. However, when taken in combination with exercise, the effect of the
15 NSAID on intestinal injury, particularly in compromised intestinal barrier function and increased intestinal permeability, is greatly enhanced.

[0010] Intestinal fatty acid binding protein (I-FABP) is a 15 kDa protein whose appearance in the plasma is a marker of enterocyte (intestinal absorptive cells) damage. Recently, van Wijk reported that ibuprofen aggravates exercise-induced small intestinal injury, specifically increasing
20 gastroduodenal and small intestinal permeability, when administered to individuals engaging in moderate exercise. This study measured the appearance of I-FABP in the peripheral plasma before, during, and after a defined exercise session (cycling). When 400 mg of ibuprofen were administered to subjects before exercise, the plasma I-FABP levels were significantly elevated relative to the plasma I-FABP levels measured from just exercise alone. The subjects also
25 experienced a significant increase in intestinal permeability, as assessed by measuring the uptake of non-metabolizable mono- and disaccharides that are ordinarily excluded by the intestinal barrier. In summary, both exercise and ibuprofen increased gut permeability. The effect of the two combined was greater than the sum of the individual effects, suggesting that the two act via different pathways, both converging on enterocyte viability and enterocyte-enterocyte barrier
30 function.

[0011] NSAIDs are commonly used by the general population to alleviate minor aches and pains and to reduce fever and inflammation. In addition, despite the risks, NSAIDs are commonly used by athletes to treat existing musculoskeletal pain and prevent any anticipated

musculoskeletal pain associated with, or induced by, exercise. For particular sports, usage among participants has been reported to be as high as 90% (T. Gorski, et al., *Br. J. Sports Med.* 45(2), 85-90 (2011); E. Taioli, *Br. J. Sports Med.* 41(7), 439-441 (2007); W.V. Thuyne, *Clin. J. Sport Med.* 18(2), 143-147 (2008).)

5 [0012] Spaces between epithelial or endothelial cells must be tightly sealed so that molecules transported from one layer to another cannot diffuse back through these spaces. Tight junctions create a barrier in epithelial and endothelial cells, regulating the movement of water and solutes through paracellular spaces. Tight junctions also function to maintain cell polarity by forming a fence to prevent intermixing of molecules in the apical membrane with those in the lateral
10 membrane. In many diseases, such as cancers or inflammatory diseases, tight junction dysfunction occurs. (See e.g., C. Foster; *Histochem Cell Biol.*; 130(1): 55-70 (July 2008).

[0013] It was reported that sphingosine-1-phosphate (S1P), a serum-borne bioactive lipid, activates tight-junction-associated protein Zonula Occludens-1 (ZO-1), which in turn plays a critical role in regulating endothelial chemotaxis and barrier integrity. (See Lee et al., *J Biol*
15 *Chem.* 29; 281(39):29190-200 (Sep 2006); Epub August 6, 2006.)

[0014] It is believed that NSAIDS likely act by two mechanisms to reduce the “tightness” of enterocyte tight junctions, consequently leading to increased intestinal permeability. Some NSAIDS sold over-the-counter in the US have the side effect of inhibiting oxidative phosphorylation. Consequently, the NSAIDS interfere with the production of cellular energy. In
20 that respect, they would be expected to exacerbate the effect of reduced blood flow to the gut observed during exercise. The reduced flow impairs the availability of oxygen, while the NSAIDs simultaneously reduce the ability of the cell to use whatever oxygen is available.

[0015] Recent evidence shows that in cultured enterocytes, S1P not only governs expression of specific tight junction proteins, but also increases the “tightness” of existing tight junctions (Greenspon et al., *Dig. Dis. Sci.* 56, 1342-1353 (2011)). The effect on junctional integrity could
25 be observed in as little as 30 minutes following exposure to S1P. S1P is created by the phosphorylation of the cellular sphingolipid, sphingosine. It is only in its phosphorylated form, as S1P, that the important governing, “signaling” effects are set into motion. The phosphorylated state of S1P is, however, under constant down-regulating activity by contiguous de-
30 phosphorylating phosphatases.

[0016] There exists a need in the art for a composition and method to treat and prevent intestinal injury, and in particular, intestinal injury caused by ingestion of an NSAID, or induced by exercise. Even more particularly, there exists a need for a composition and method to treat

and prevent exercise-induced intestinal injury for those who frequently consume NSAIDs prior to, during, or immediately following exercise to reduce and/or prevent exercise-associated stiffness or pain. Preferably such a method is non-toxic, non-hazardous, and without significant adverse side effects. There is also a need for novel methods and compositions for regulating tight junctions, which can be used for preventing or treating diseases related to tight junction dysfunction.

BRIEF SUMMARY OF THE INVENTION

[0017] The present invention provides methods and compositions for treating or preventing intestinal injury or symptom thereof, including intestinal injury induced or aggravated by ingestion of a nonsteroidal anti-inflammatory drug (NSAID), exercise-induced intestinal injury, and exercise-induced intestinal injury aggravated by ingestion of an NSAID to treat or prevent musculoskeletal pain associated with exercising. According to embodiments of the present invention, administration of a glycerophosphate salt, and particularly administration of calcium glycerophosphate, to a subject is an effective method for reducing, alleviating, and/or preventing intestinal injury or symptom thereof in the subject.

[0018] In one general aspect, the present invention relates to a method of treating or preventing an intestinal injury or symptom thereof in a subject, the method comprising administering to the subject a composition comprising a therapeutically effective amount of a glycerophosphate salt such that intestinal injury or symptom thereof is treated or prevented. In one embodiment, the intestinal injury or symptom thereof is exercise-induced and the subject engages in exercise. Preferably, the composition is administered orally or nasally, more preferably orally.

[0019] In another general aspect, the present invention relates to a method of treating or preventing an intestinal injury or symptom thereof aggravated by a nonsteroidal anti-inflammatory drug (NSAID) in a subject, wherein the subject is administered the NSAID, the method comprising administering to the subject a composition comprising a therapeutically effective amount of a glycerophosphate salt such that the intestinal injury or symptom thereof aggravated by the NSAID is treated or prevented. Preferably, the composition is administered orally or nasally, more preferably orally. In one embodiment, the intestinal injury or symptom thereof is exercise-induced and the subject engages in exercise.

[0020] According to embodiments of the present invention, administering a therapeutically effective amount of a glycerophosphate salt, and preferably calcium glycerophosphate, to a

subject results in the reduction, alleviation, or prevention of intestinal injury and/or one or more symptoms thereof, such as those that are exercise-induced, as compared to the intestinal injury and/or symptoms thereof that result when the subject is not administered a glycerophosphate salt. The glycerophosphate salt, and preferably calcium glycerophosphate, is also effective in
5 reducing, alleviating or preventing intestinal injury and/or one or more symptoms thereof further aggravated by ingestion of an NSAID, such as those that are exercise-induced, as compared to the intestinal injury and/or symptoms thereof that may result when the subject is not administered the glycerophosphate salt.

[0021] The present invention also relates to a novel enteric coated composition comprising a
10 therapeutically effective amount of a glycerophosphate salt, and methods of treating or preventing intestinal injury in a subject, increasing tight junction integrity in a subject, and treating or preventing diseases related to tight junction dysfunction in a subject, comprising administering to the subject an enteric coated composition according to the invention, such as a delivery site-specific composition.

[0022] In one general aspect, the present invention provides an enteric coated composition
15 comprising a therapeutically effective amount of a glycerophosphate salt. According to embodiments of the present invention, an enteric coated composition comprises:
a core comprising a therapeutically effective amount of a glycerophosphate salt; and
an enteric coating surrounding the core,

[0023] In a preferred embodiment, the enteric coating prevents release of the
20 glycerophosphate salt from the core until the composition is assured of substantially reaching at least the jejunal/ileal sectors of the small intestine, as well as the large intestine, of the subject.

[0024] In another general aspect, the present invention also relates to method of increasing
25 tight junction integrity in a subject in need thereof, comprising administering to the subject a composition comprising an effective amount of glycerophosphate.

[0025] In another general aspect, the invention also relates to methods of treating or
preventing a disease related to tight junction dysfunction in a subject in need thereof, comprising administering to the subject a composition comprising an effective amount of glycerophosphate.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The following detailed description of the invention will be better understood when
30 read in conjunction with the appended drawings. For the purpose of illustrating the invention, there are shown in the drawings embodiments which are presently preferred. It should be

understood, however, that the invention is not limited to the embodiments shown. In the drawings:

[0027] Fig. 1 is a graph of baseline mannitol flux versus time in the presence and absence of increasing concentrations of CGP;

5 [0028] Fig. 2 are graphs showing the effect of CGP on trans-epithelial electrical resistance (TEER) in the presence and absence of increasing concentrations of CGP;

[0029] Fig. 3 is a graph showing the effect of CGP on baseline production of sphingosine 1-phosphate in Caco-2 cells;

10 [0030] Fig. 4 is a graph showing the increase in mannitol penetration in Caco-2 cells four hours after the administration of calcium glycerophosphate (CGP) to the cells;

[0031] Fig. 5 are graphs of transepithelial mannitol flux during hypoxia;

[0032] Fig. 6 are graphs of TEER during hypoxia;

[0033] Fig. 7 are graphs showing the effect of CGP on transepithelial mannitol flux induced by cytomix; and

15 [0034] Fig. 8 are graphs showing the effect of CGP on transepithelial electrical resistance during cytomix ((TNF- α), (IFN- γ), and (IL- β)) treatment.

DETAILED DESCRIPTION OF THE INVENTION

[0035] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention pertains. All publications and patents referred to herein are incorporated by reference.

20 Discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is for the purpose of providing context for the present invention. Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

25 [0036] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

[0037] As used herein, the term “glycerophosphate salt” refers to any salt comprising a glycerophosphate moiety and a non-toxic cation. Non-limiting examples of non-toxic cations include calcium, sodium, potassium, and magnesium. Glycerophosphate salts suitable for use in
30 the present invention include, but are not limited, to calcium glycerophosphate, sodium glycerophosphate, potassium glycerophosphate, and magnesium glycerophosphate. Preferably, the glycerophosphate salt is calcium glycerophosphate.

[0038] As used herein, the term “calcium glycerophosphate” or “CGP” refers to a compound having a molecular formula of $C_3H_7CaO_6P$ and a molecular weight of 210.04 in anhydrous form. “CGP” can also exist as a hydrate, including the monohydrate and dihydrate forms. “CGP” is also known as 1,2,3-propanetriol, mono(dihydrogen phosphate) calcium salt (1:1), calcium
5 glycerinophosphate, calcium phosphoglycerate and NEUROSIN®. Three CGP isomers exist, namely β -glycerophosphoric acid calcium salt $((HOCH_2)_2CHOPO_3Ca)$ and D(+) and L(-)- α -glycerophosphoric acid calcium salt $(HOCH_2CH(OH)CH_2OPO_3Ca)$. Any one isomer, or any combination of two or more isomers, may be used as the CGP according to this invention.

[0039] CGP can be synthesized using methods known in the art. CGP can also be obtained
10 from various commercial sources. The commercially available CGP preparations include, but are not limited to, those available from AkPharma Inc. (Pleasantville, NJ 08232), Astha Laboratories Pvt, Ltd, (B-4, Industrial Estate, Sanathnagar, Hyderabad-18, India), Seppic Inc. (30 Two Bridges Road, Fairfield, NJ 07004), and numerous other manufacturers and distributors around the world.

[0040] As used herein, the term “nonsteroidal anti-inflammatory drug” or “NSAID” refers to
15 a class of compounds that have analgesic (pain reducing), antipyretic (fever reducing), and/or anti-inflammatory effects. Examples of NSAIDs include, but are not limited to, ibuprofen, aspirin, naproxen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin, indomethacin, sulindac, etodolac, diclofenac, piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam, and isoxicam, and pharmaceutically acceptable salts thereof.

[0041] As used herein, the term “exercise” refers to any form of physical activity. In one
20 embodiment, exercise can refer to any activity aimed at improving flexibility, or the range of motion of muscles and joints, such as stretching. In another embodiment, exercise can refer to any aerobic activity aimed at increasing cardiovascular endurance, including, but not limited to, cycling, swimming, walking, rowing, running, hiking, and tennis. In yet another embodiment,
25 exercise can refer to any anaerobic activity aimed at increasing short-term muscle strength, such as, for example, weight training, interval training, tennis, football at all age and competitive levels, soccer, sprinting, or any other physically demanding sport or activity. Although a method of the present invention can treat intestinal injury caused by any form of exercise in view of the present disclosure, intestinal injury may be more likely to occur following aerobic exercise as
30 compared to anaerobic exercise because the oxygen demand in aerobic exercise is greater, and thus the need for treatment following aerobic exercise may also be greater.

[0042] As used herein, the term “subject” means any animal, preferably a mammal, most preferably a human, to whom will be or has been administered compositions or compounds

according to embodiments of the present invention. Examples of mammals include, but are not limited to, cows, horses (most particularly race horses), sheep, pigs, cats, dogs, mice, rats, cats, dogs, rabbits, guinea pigs, monkeys, humans, etc., most preferably humans.

[0043] As used herein, the term “intestine” refers to the intestinal tract. The intestinal tract includes the small intestine and the large intestine. The small intestine is composed of the duodenum (i.e., the uppermost portion proximal to the stomach), the jejunum (i.e. the middle portion/segment), and the ileum (i.e., the final section prior to the large intestine). As used herein, “junction between the duodenum and jejunum portions” refers to the portion of the small intestine wherein the duodenum segment meets the jejunum segment. As used herein, “junction between the duodenum and jejunum portions” refers to the portion of the small intestine wherein the duodenum segment meets the jejunum segment.

[0044] The intestinal barrier to intestinal cell permeability is dependent on the formation of tight junctions between adjacent enterocytes, i.e., intestinal cells. Tight junctions are composed of strands of proteins (principally claudins and occludins) that girdle the cell near the intestinal lumen. The junctional strands of one cell bind to those on an adjacent cell, forming a seal between the cells, in much the same way that tongue-and-groove joints hold floorboards together. The “tightness” of the tight junction between cells is a direct function of the number of strands, and can be influenced by the oxidative state of the cell as well as soluble factors on the surface of the cell.

[0045] Substances move across the intestinal barrier by either the transcellular (across the cell) or paracellular (between the cells) routes. By definition, substances that travel between cells are able to traverse tight junctions. The rate at which they do so depends on both the molecular weight of the substance and the “tightness” of the tight junctions.

[0046] As used herein, the term “intestinal injury” refers to damage to the intestine, and in particular damage to the enterocytes (intestinal cells) themselves. Enterocytes are the predominant cells in the small and large intestinal mucosa responsible for the final digestion and absorption of nutrients, electrolytes, and water. “Intestinal injury” also refers to damage to the junctions between intestinal cells, i.e., the enterocyte-enterocyte tight junctions, such that normal barrier intestinal function is impaired, diminished, or has deteriorated. The scope of the term “intestinal injury” as used herein is intended to further encompass increased intestinal permeability as a result of compromised enterocyte-enterocyte tight junctions, consequently compromising the intestinal epithelial barrier integrity and permitting diffusion of substances across the intestinal barrier that would ordinarily be excluded. “Intestinal injury” as used herein

also refers to symptoms, and the onset of symptoms, of intestinal injury, including, but not limited to, epigastric pain, flatulence, dyspepsia (indigestion), and belching.

[0047] As used herein, the terms “increased gut permeability” and “increased intestinal permeability” refer to a decrease in the “tightness” of the tight junctions between intestinal cells.

5 [0048] As used herein, a “therapeutically effective amount” means an amount of a glycerophosphate salt that is effective in reducing, alleviating, causing intestinal injury to develop to a lesser extent, or preventing an intestinal injury. A “therapeutically effective amount” is also an amount of a glycerophosphate salt that is effective in reducing, alleviating, causing to develop to a lesser extent, or preventing any symptoms associated with, or indicative of, an intestinal
10 injury.

[0049] As used herein, the terms “treating,” “treat,” or “treatment” refer to the administration of an amount of a glycerophosphate salt to a subject that is effective in reducing, alleviating, or preventing intestinal injury and/or one or more symptoms associated with intestinal injury in a subject.

15 [0050] As used herein the terms “preventing,” “prevent,” or “prevention” refer to the administration of a therapeutically effective amount of a glycerophosphate salt to a subject before the onset of symptoms associated with intestinal injury, such that any intestinal injury, or associated symptoms, will be prevented altogether, will be time-delayed as to occurrence, or may still occur, but will do so to a lesser extent than in the absence of a glycerophosphate salt.

20 [0051] Embodiments of the present invention comprise methods of treating or preventing intestinal injury in a subject. Because increased intestinal permeability can be the consequence of exercise-induced blood flow diversion during exercise from the splanchnic circulation (i.e. blood flow to the gut) to meet increased musculoskeletal oxygen demand, exercise alone can cause intestinal injury. The intestinal injury induced by exercise can be further aggravated or
25 exacerbated by ingestion of an NSAID. Thus, embodiments of the present invention also comprise methods of treating or preventing exercise-induced intestinal injury in a subject, methods of treating or preventing intestinal injury aggravated by an NSAID in a subject, and methods of treating or preventing exercise-induced intestinal injury aggravated by the ingestion of an NSAID in a subject.

30 [0052] As used herein the phrase “exercise-induced intestinal injury” refers to intestinal injury, or symptoms of intestinal injury, caused by exercise such that the resulting intestinal injury or symptoms of intestinal injury are greater than the intestinal injury, or symptoms of intestinal injury that would have resulted in the absence of exercise. As used herein, the phrase

“exercise-induced intestinal injury aggravated by an NSAID” refers to intestinal injury, or symptoms of intestinal injury, caused by exercise and exacerbated by, or increased by, ingestion of an NSAID, such that the resulting intestinal injury, or symptoms of intestinal injury, are greater than the intestinal injury, or symptoms of intestinal injury, that would have resulted in the absence of exercise and the ingestion of an NSAID.

Methods of Treating or Preventing Intestinal Injury or Symptoms

[0053] According to embodiments of the present invention, a method of treating or preventing intestinal injury or a symptom thereof in a subject comprises administering to the subject a therapeutically effective amount of a glycerophosphate salt. In one embodiment, the intestinal injury or symptom thereof to be treated or prevented is exercise-induced. In another embodiment, the intestinal injury or symptom thereof to be treated or prevented is exercise-induced and further aggravated by ingestion of an NSAID.

[0054] Thus, according to embodiments of the present invention, a subject to be administered an effective amount of a glycerophosphate salt can be one who is engaging in exercise, such that the intestinal injury to be treated or prevented is exercise induced. A subject to be administered an effective amount of a glycerophosphate salt can also be one who ingests an NSAID, such that the intestinal injury to be treated or prevented is induced by the NSAID. A subject to be administered a glycerophosphate salt according to the invention can further be one who is engaging in exercise and ingesting an NSAID, such as to alleviate current or anticipated exercise-associated musculoskeletal pain. Non-limiting examples of exercise-associated musculoskeletal pain that can be treated by the intake of an NSAID include inflammation of the joints, such as in knees or elbows; joint injuries, such as sprains; inflammation of the tendons (tendonitis); strained or torn ligaments, such as a torn anterior cruciate ligament (ACL); tibial stress syndrome (shin splints); plantar fasciitis; and strained hamstring, quadriceps, gluteal, bicep, or tricep muscles (pulled muscles). Exercise-associated musculoskeletal pain that can be treated by intake of an NSAID also includes simple muscle and joint aches post-exercise, with no specific trauma experienced or indicated. For the purposes of this disclosure, the actual effectiveness of the NSAID in producing relief of the perceived or anticipated pain at any locale, is not of immediate concern; only the fact that the exerciser ingest such NSAID at or near the time of such exercise, putting the intestinal cells at specific risk, thereby.

[0055] According to the present invention, a subject's ingestion of an NSAID and engagement in exercise need not be simultaneous, but only contemporaneous or concurrent, such

that an effective amount of an NSAID is present in the subject in imminent anticipation of, or at the initiation of exercise, and thus has the potential to exacerbate or aggravate any intestinal injury induced by the exercise itself from the moment of initiation of the exercise. For example, an NSAID, such as ibuprofen, can be ingested 60 minutes prior to engaging in exercise, such as running.

[0056] In one embodiment of the present invention, the administration of a therapeutically effective amount of a glycerophosphate salt results in a clinically observable beneficial effect in the reduction, alleviation, or prevention of one or more symptoms of intestinal injury, such that symptoms are about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or less than the symptoms that would have resulted from intestinal injury, had the subject not received a therapeutically effective amount of the glycerophosphate salt.

[0057] In another embodiment of the present invention, the administration of a therapeutically effective amount of a glycerophosphate salt results in a clinically observable beneficial effect in the reduction, alleviation, or prevention of one or more symptoms of intestinal injury aggravated by an NSAID, such that symptoms are about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or less than the symptoms that would have resulted from ingestion of an NSAID, had the subject not received a therapeutically effective amount of the glycerophosphate salt.

[0058] In yet another embodiment of the present invention, the administration of a therapeutically effective amount of a glycerophosphate salt results in a clinically observable beneficial effect in the reduction, alleviation, or prevention of one or more symptoms of exercise-induced intestinal injury, such that symptoms are about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or less than the symptoms that would have resulted from exercise alone, had the subject not received a therapeutically effective amount of the glycerophosphate salt.

[0059] And in yet another embodiment of the present invention, the administration of a therapeutically effective amount of a glycerophosphate salt results in a clinically observable beneficial effect in the reduction, alleviation, or prevention of one or more symptoms of exercise-induced intestinal injury aggravated by an NSAID, such that symptoms are about 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10% or less than the symptoms that would have resulted from exercise and ingestion of an NSAID, had the subject not received a therapeutically effective amount of the glycerophosphate salt.

[0060] In yet another embodiment of the present invention, the clinically observable beneficial effect can be a situation wherein intestinal injury, intestinal injury aggravated by an NSAID, exercise-induced intestinal injury or exercise-induced intestinal injury aggravated by an

NSAID, and/or one or more symptoms thereof, is prevented from further aggravation or development, or subsequently develops to a lesser degree, than would have resulted had the subject not received a therapeutically effective amount of a glycerophosphate salt.

5 [0061] In yet another embodiment of the present invention, the clinically observable beneficial effect can be a situation where intestinal injury, intestinal injury aggravated by an NSAID, exercise-induced intestinal injury or exercise-induced intestinal injury aggravated by an NSAID, and/or one or more symptoms thereof, is prevented from occurring, such that the subject does not experience any intestinal injury or symptoms of intestinal injury that would have resulted had the subject not received a therapeutically effective amount of a glycerophosphate salt.

10 [0062] In a preferred embodiment, the glycerophosphate salt administered to the subject is calcium glycerophosphate.

15 [0063] In view of the present disclosure, standard procedures can be performed to evaluate the effect of administration of a glycerophosphate salt to a subject, thus allowing a skilled artisan to determine the effective amount of the glycerophosphate to be administered to a subject. The clinically beneficial effect of the glycerophosphate salt can be readily apparent to the subject. For example, a subject administered an effective amount of a glycerophosphate salt who also ingested an NSAID prior to exercising can subsequently experience no indigestion, epigastric pain, or flatulence during and after exercise as compared to the amount of indigestion, epigastric pain, or flatulence experienced had the glycerophosphate salt not been administered. Symptoms directly observable to the subject can also include constipation or diarrhea or symptoms of inflammation, such as achy joints, muscle tenderness, etc. The symptoms to be evaluated can also resemble the musculoskeletal symptoms normally anticipated following vigorous exercise, such as muscle or joint pain etc.

20 [0064] The clinically beneficial effect of a glycerophosphate salt can also be determined by a clinical assay to evaluate the efficacy of the glycerophosphate salt in preserving intestinal barrier integrity, and thus treating or preventing intestinal injury. In this case, the clinically beneficial effect can be determined objectively, and the clinically beneficial effect may not necessarily be directly observable or readily apparent to the subject. For example, enterocyte damage, such as that which might result from impaired oxygen availability and/or utilization, liberates intestinal fatty acid binding protein (I-FABP) from the cell. I-FABP then enters circulation, and can be detected in plasma. Thus, plasma I-FABP is a marker of intestinal cell damage and I-FABP levels can be readily measured, such as by enzyme linked immunoabsorbent assay (ELISA), to

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determine the effect of the glycerophosphate salt on reducing intestinal injury. Another illustrative example of a clinical assay for evaluating intestinal injury and the efficacy of a glycerophosphate salt in preventing or reducing intestinal injury is a multiple sugar assay as described in *J. Chromatog. B Analyt. Tech. Biomed. Life Sci.*, 879(26), 2794-2801 (2011),
5 incorporated herein by reference. The multiple sugar assay measures intestinal cell permeability by measuring the uptake and urinary excretion of non-metabolizable mono- and disaccharides. These sugars are taken up by paracellular (between cells) routes, but only when the tightness of the enterocyte-enterocyte junctions is reduced.

[0065] Dosages of a glycerophosphate salt administered to a subject to provide a
10 therapeutically effective amount of the glycerophosphate salt are not limited to a particular value. One of ordinary skill in the art will recognize that the therapeutically effective amount of a glycerophosphate salt necessary to observe a clinically beneficial effect will depend on additional factors such as the weight, age, gender, etc. of a subject to be treated, as well as their intestinal health to begin with, and will be readily able to determine the appropriate dosage that provides a
15 therapeutically effective amount. Additional factors to be considered in determining the dosing regimen and therapeutically effective amount of a glycerophosphate salt administered to a subject according to the present invention include, but are not limited to, the sensitivity of the subject to NSAIDs and the intensity and regularity of exercise engaged in by the subject. For example, to
20 treat or prevent exercise induced intestinal injury aggravated by ingestion of an NSAID in a subject, the subject can be administered CGP. Dosage of CGP relative to an NSAID can cover a wide range, and preferably CGP is administered in a mole ratio of CGP:NSAID ranging from 0.1:1 to 10:1. As an illustrative example, if the NSAID to be used is ibuprofen, a typical dosage would be a 1:1 ratio of CGP:ibuprofen by weight, in view of the fact that CGP and ibuprofen have nearly the same molecular weight (MW CGP= 210.04; MW ibuprofen= 206.29 g/mol). In
25 this case, 200 mg of CGP can be administered for a 200 mg dose of ibuprofen. Likewise, 400 mg of CGP can be administered for a 400 mg dose of ibuprofen, etc. If aspirin (MW= 189 g/mol) is administered as the NSAID, where the recommended dosage of aspirin can be 650 mg, the corresponding dosage of CGP can be 800 mg. For other NSAIDs, the dose of CGP would be similarly matched with the recommended or typical dose of the NSAID. When administered to a
30 subject to treat or prevent exercise-induced intestinal injury in the absence of an NSAID, the dose of CGP, or other glycerophosphate salt, can be the same as that which would be administered had an NSAID also been ingested.

[0066] The glycerophosphate salt and NSAID can be administered in a single composition, such as a combination tablet or capsule, or they can be administered in separate compositions, such as two separate tablets or capsules. In either case, both the glycerophosphate salt and NSAID need not be ingested simultaneously, but must be ingested contemporaneously or concurrently such that both the glycerophosphate salt and NSAID contact the vulnerable intestinal cells contemporaneously with one another.

[0067] According to one embodiment of the present invention, a therapeutically effective amount of a glycerophosphate salt is administered orally to a subject in solid form or liquid form, such as a powder or a liquid mixture. When administered orally as a solid, the glycerophosphate salt can be, for example, in the form of a powder, tablet, capsule, caplet, gelcap, or liquid gel. When administered orally as a liquid, the glycerophosphate salt can be, for example, in the form of an emulsion, solution, suspension, syrup, or elixir.

[0068] According to another embodiment of the present invention, a therapeutically effective amount of a glycerophosphate salt is administered nasally to a subject as a solid powder or liquid mixture in a nasal spray/inhalant mixture. When administered nasally as a nasal spray/inhalant mixture, the glycerophosphate salt can be in the form of particles, or as a liquid emulsion, solution syrup, or elixir dissolved/suspended in an aqueous carrier solution/suspension. In this embodiment, delayed-release is not necessary.

[0069] In a preferred embodiment, the glycerophosphate salt is administered orally. While oral administration is the preferred method because nasal administration is not as direct of a route to the intestines, owing to substantial absorption at the nasal membrane level, some of the nasally administered product can easily be swallowed via rear area upper respiratory drainage down through the esophagus, thereby being routed directly to the intestinal tract, although via such routing there is no delayed-release for that portion which proceeds gastrointestinally.

[0070] According to another embodiment of the present invention, the glycerophosphate salt can be administered orally in combination with a sports-directed food or drink. As used herein, the term "sports-directed food or drink" refers to any beverage or food item specifically marketed to athletes for enhancing performance of, or recovery from, exercise, training, athletic performance, or any activity related to exercise or athletic performance. Non-limiting examples of sports-directed food and drink include energy bars, protein powders, protein shakes, and sports drinks, particularly those designed to aid in hydration after exercise or replace electrolytes. The glycerophosphate salt can be dissolved, or suspended in, a sports-directed drink and orally administered to a subject upon ingestion of the sports-directed drink by the subject. The

glycerophosphate salt can also be combined with a sports-directed food and thus administered orally to a subject upon ingestion of the sports-directed food by the subject. For example, a CGP powder can be mixed with a protein powder, or disintegrated or suspended in a protein bar.

[0071] Compositions comprising a therapeutically effective amount of a glycerophosphate salt for use in the present invention can be formulated using any method known to those skilled in the art in view of the present disclosure. To prepare the compositions for administration to a subject, the glycerophosphate salt is mixed with a pharmaceutically acceptable carrier according to conventional pharmaceutical compounding techniques. Compositions for use in the present invention can comprise a therapeutically effective amount of a glycerophosphate salt that is about 10-90% glycerophosphate salt, preferably 50-90%, and most preferably 75-90% of the total weight of the composition. While the active portion of the composition preferably contains 100% of the glycerophosphate salt, the percentage of glycerophosphate in the entire formulated compound will be a function of the further weight of the protective coating involved to ensure that the active salts reaches the appropriate sections of the small and large intestines. The amount by weight of glycerophosphate salt will depend on the specific cation of the glycerophosphate salt. Preferably, the core comprises at least about 95% of the composition, with the protective coating comprising the balance. However, these percentages are not limiting and may be determined by routine experimentation.

[0072] Pharmaceutically acceptable carriers can include one or more excipients such as binders, suspending agents, emulsifying agents, wetting agents, lubricants, flavorants, sweeteners, preservatives, dyes, and coatings. Carriers can take a wide variety of forms depending on the form of preparation desired for administration. For liquid oral preparations, for example, suspensions, elixirs and solutions, suitable carriers and additives include water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like. For solid oral preparations, for example, powders, capsules, caplets, gelcaps and tablets, suitable carriers and additives include starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like. For nasal sprays/inhalant mixtures, the aqueous solution/suspension can comprise water, glycols, oils, emollients, stabilizers, wetting agents, preservatives, aromatics, flavors, and the like as suitable carriers and additives.

[0073] The solubility of CGP in water is approximately 1 gram in 100 mL. Thus, a solution/suspension of CGP can be prepared for oral administration according to the invention, for example, by dissolving/disintegrating and suspending CGP powder or tablets in water. Where a suspension is deemed acceptable, a concentration greater than 1% CGP can be used, and kept in

suspension by use of any number of common, known stabilizers including, but not limited to, agar, sodium carboxymethylcellulose gum, guar gum, etc. In a particular embodiment, CGP powder or tablets can be dissolved in a sports-directed drink, or disintegrated, suspended, or mixed into a sports-directed food. CGP can also be formulated in an extended release form suitable for several hours for use in the present invention. Methods are known to those skilled in the art to manufacture the extended release dosage form. A typical administration of a nasal spray could be from one spray per nostril daily to two sprays per nostril more than one time per day, as needed, depending on the amount of NSAID ingested by the subject. Compositions comprising other glycerophosphate salts, such as sodium, magnesium, or potassium glycerophosphate can be prepared similar to CGP compositions.

[0074] According to embodiments of the present invention, a glycerophosphate salt administered to treat or prevent intestinal injury can be administered to a subject at any time point. For example, a glycerophosphate salt can be administered once or twice daily to treat or prevent intestinal injury, or even more frequently, as desired, with no ill effects.

[0075] According to embodiments of the present invention, a glycerophosphate salt administered to treat or prevent intestinal injury aggravated by an NSAID can be administered to a subject at any point provided that the therapeutic effects of the glycerophosphate salt overlap with administration of the NSAID and its effects in aggravating intestinal injury, such that a clinically beneficial effect can be observed. The glycerophosphate salt can be administered, for example, simultaneously with the NSAID, immediately prior to ingestion of the NSAID, or immediately after ingestion of the NSAID.

[0076] According to embodiments of the present invention, a glycerophosphate salt administered to treat or prevent exercise-induced intestinal injury can be administered to a subject at any point provided that the therapeutic effects of the glycerophosphate salt overlap with the onset or persistence of exercise-induced intestinal injury, such that a clinically beneficial effect can be observed. Preferably, the glycerophosphate salt is administered immediately prior to the initiation of exercise, during exercise, or immediately following exercise.

[0077] According to embodiments of the present invention, a glycerophosphate salt administered to treat or prevent exercise-induced intestinal injury aggravated by an NSAID can be administered to a subject at any point provided that the therapeutic effects of the glycerophosphate salt overlap with administration of the NSAID and its effects in aggravating exercise-induced intestinal injury, such that a clinically beneficial effect can be observed. The glycerophosphate salt can be administered, for example, after ingestion of an NSAID but prior to

the initiation of exercise, or the glycerophosphate salt can be administered after ingestion of an NSAID but during the course of exercise. The glycerophosphate salt can be administered prior to both the ingestion of an NSAID and exercise. The glycerophosphate salt can also be administered after ingestion of an NSAID, but after the completion of exercise, or simultaneously with the ingestion of an NSAID prior to the initiation of exercise.

[0078] Because glycerophosphate salts, and particularly CGP, are non-toxic, non-hazardous, and have no known side-effects, the number of times the glycerophosphate salt can be administered is not limited in any way. Thus, the glycerophosphate salt can also be administered multiple times per day, such as once, twice or three times or more per day. For example, when treating or preventing exercise-induced intestinal injury, a glycerophosphate salt, such as CGP, can be administered multiple times, such as before and after exercising, or before, during and after exercising. If the object is to prevent intestinal injury aggravated by an NSAID, a glycerophosphate salt can be administered each time an NSAID is ingested. When the object is to prevent any anticipated aggravated exercise-induced intestinal injury by an NSAID and associated symptoms thereof, it is preferable for the glycerophosphate salt to be administered prior to, or simultaneously with, ingestion of the NSAID. The situations presented above are intended to be non-limiting examples and it is to be understood that one of ordinary skill in the art will recognize when administration of CGP or other glycerophosphate salt, and particularly administration of CGP or other glycerophosphate salt relative to the ingestion of an NSAID, and even more particularly administration of CGP or other glycerophosphate salt relative to the ingestion of an NSAID and engagement in exercise, will yield a clinically beneficial effect according to the invention.

[0079] Compositions comprising a therapeutically effective amount of a glycerophosphate salt for use in the present invention can optionally comprise additional therapeutic additives, including NSAIDs, such as those discussed above. Thus, in a particular embodiment of the present invention, a glycerophosphate salt and an NSAID can be administered to a subject simultaneously as part of the same composition. Preferably, the glycerophosphate salt is CGP. A CGP and NSAID combination composition for use in the present invention preferably comprises an amount of CGP by weight that is about 10% to about 1000% of the weight of the NSAID in the mixture of CGP and NSAID, preferably 30% to 70%, more preferably 40% to 60%, and most preferably 50% of the weight of the NSAID in the composition. When taken simultaneously with an NSAID, but not part of a mutual composition with an NSAID, the weight of the CGP is preferably 1:1 relative to the weight of the NSAID. In a preferred embodiment, the NSAID is

ibuprofen. As an illustrative but non-limiting example, a composition comprising CGP and ibuprofen can be administered to a subject 60 min prior to engaging in exercise, such as running, to treat and/or prevent exercise-induced intestinal injury according to the invention.

[0080] Individuals who chronically ingest NSAIDs prior to, during, or immediately following exercise to reduce exercise associated musculoskeletal pain can be athletes following strict training regimens, requiring intense exercise on an almost daily basis. For such subjects, extended release formulations of the glycerophosphate salt for the treatment or prevention of exercise-induced intestinal injury aggravated by NSAIDs can be desirable. Use of an extended release formulation of a glycerophosphate salt could limit the number of times administration of the glycerophosphate salt is required without sacrificing therapeutic efficacy. However, as discussed above, glycerophosphate salts, and particularly CGP, are non-toxic, non-hazardous, and have no known adverse side effects, thus subjects prescribed an extended release formulation of a glycerophosphate salt could optionally supplement their dosing regimens with standard glycerophosphate salt formulations to maximize the therapeutic effect. Preferably, an extended release or delayed release formulation for use in the present invention comprises CGP.

Methods for Increasing Tight Junction Integrity and Treating or Preventing Diseases Related to Tight Junction Dysfunction

[0081] It is surprisingly discovered in the present invention that a glycerophosphate, such as calcium glycerophosphate (CGP), increases tight junction integrity and decreases transepithelial permeability.

[0082] Accordingly, a general aspect of the present invention relates to a method of increasing tight junction integrity in a subject in need thereof, comprising administering to the subject a composition comprising an effective amount of glycerophosphate, such as calcium glycerophosphate (CGP). Determination of the effective amount of glycerophosphate, the effectiveness of administration, the method of administration, and the components in the composition have been described previously with respect to method of treating intestinal injury or symptom thereof.

[0083] Another general aspect of the invention relates to a method of treating or preventing a disease related to tight junction dysfunction in a subject in need thereof. The method comprises administering to the subject a composition comprising an effective amount of glycerophosphate, such as calcium glycerophosphate (CGP). Determination of the effective amount of glycerophosphate, the effectiveness of administration, the method of administration, and the

components in the composition have been described previously with respect to method of treating intestinal injury or symptom thereof.

[0084] Any disease related to tight junction dysfunction can be prevented or treated by the present method.

5 [0085] In one embodiment of the present invention, the disease related to tight junction dysfunction is a cancer, such as breast cancer, e.g., invasive ductal cancer; prostate cancer, e.g., prostatic adenocarcinomas; thyroid neoplasma, follicular adenoma; gastroesophageal reflux disease, e.g., Barrett's esophagus (dysplasia); lung cancer, e.g., basaloid squamous carcinoma; etc. (See C. Foster; *Histochem Cell Biol.*; 130(1): 55–70 (July 2008), the disclosures of which is
10 incorporated by reference herein in its entirety.)

[0100] In another embodiment of the present invention, the disease related to tight junction dysfunction is an inflammatory disease, including but not limited to, inflammatory bowel disease (IBD), such as Morbus Crohn collagenous colitis; celiac, sprue, Crohn's disease, ulcerative colitis, multiple sclerosis; hereditary diseases, such as hereditary deafness, familial
15 hypomagnesemia, neuroinflammation (see Grin'kina et al., PLoS ONE 7(5): e36475. doi:10.1371/journal.pone.0036475 (2012), the disclosure of which is incorporated by reference herein in its entirety), cystic fibrosis; vision loss, such as diabetic eye disease: diabetic retinopathy; viral infection, such as retroviral infection (hydrocephalus, encephalitis); bacterial toxins, such as *Clostridium perfringens* enterotoxin; a disease in lung tissue integrity; a disease in
20 lymphocyte trafficking (see Mandala et al., *Science* 296, 346 (2002), the disclosure of which is incorporated by reference herein in its entirety, or other immunological diseases (see Rosen et al., *Nature Reviews Immunology* 5, 560-570 (July 2005), the disclosure of which is incorporated by reference herein in its entirety), etc. In a preferred embodiment, the present invention relates to a method of preventing or treating an inflammatory bowel disease (IBD) in a subject in need
25 thereof, comprising administering to the subject a composition comprising an effective amount of glycerophosphate, such as calcium glycerophosphate (CGP). More preferably, the IBD is Morbus Crohn collagenous colitis or Crohn's disease, which has diarrhea as a leading symptom and is chiefly attributed to epithelial barrier dysfunction that results in an increased loss of solutes in the "leaking flux diarrhea."

30 [0101] While not wishing to be bound by theories, it is believed that glycerophosphate, such as calcium glycerophosphate (CGP), elevates sphingosine-1-phosphate (S1P) concentrations and/or activity in epithelial or endothelial cells, consequently decreasing transepithelial permeability. Without wishing to be bound by theory, it is also believed that glycerophosphate salts act, at least

in part, by inhibiting the action of phosphatases, including those that dephosphorylate S1P, resulting in the glycerophosphate salts mediating the formation and longevity of S1P and increasing S1P's availability to preserve junction integrity in a gut challenged by NSAIDs and exercise induced ischemia.

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Enteric Coated Compositions

[0102] The present invention also provides an enteric coated composition comprising a therapeutically effective amount of a glycerophosphate salt. According to embodiments of the present invention, an enteric coated composition according to the invention is a delayed-release formulation that can be used to treat intestinal injury, intestinal injury aggravated by an NSAID, exercise-induced intestinal injury, and exercise-induced intestinal injury further aggravated by an NSAID. Thus, embodiments of the present invention also encompass methods of treating or preventing intestinal injury or symptoms thereof in a subject comprising administering to the subject an enteric coated composition according to the invention. Embodiments of the present invention also encompass methods of increasing tight junction integrity in a subject and methods for treating or preventing a disease related to tight junction dysfunction in a subject comprising administering to the subject an enteric-coated composition according to the invention.

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[0103] According to embodiments of the present invention, an enteric coated composition comprises: (i) a core comprising a therapeutically effective amount of glycerophosphate salt; and (ii) an enteric coating surrounding the core. In a preferred embodiment, the glycerophosphate salt is calcium glycerophosphate.

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[0104] As used herein, the term "enteric coated composition" refers to a composition having an enteric coating that surrounds and encases the exterior of a core of the composition, wherein the core comprises the therapeutically or pharmacologically active compound. As used herein, the term "core" refers to the component of the enteric coated composition that comprises the therapeutically active ingredient. A core employed in an enteric coated composition according to the invention is preferably formulated in a solid dosage form for oral administration, such as, for example, a powder, tablet, capsule, caplet, gelcap, liquid gel, or pellet etc. Because the core of a composition according to the invention is surrounded and encased by an enteric coating, the core comprises an interior layer or portion of the composition.

[0105] As used herein, the term "enteric coating" refers to a layer that surrounds and encases the core of a composition, and is the outermost layer of the composition. Preferably, the enteric

coating completely surrounds and encases the core, such that the core is not exposed to any bodily fluids upon administration to a subject (e.g., saliva, gastrointestinal juices) until the enteric coating has dissolved or disintegrated. The enteric coating prevents the therapeutically or pharmacologically active ingredient in the core of the composition from being released until the enteric coating is degraded, dissolved or broken down, but permits release of the therapeutically active ingredient from the core once the enteric coating is dissolved or degraded, or is beginning to dissolve or degrade. Preferably, the enteric coating is not degraded, dissolved, or broken down, and thus does not permit release of the therapeutically active ingredient from the core until the composition reaches the intestine, and more preferably it permits the quantitative release of the therapeutically active ingredient from the core to at least the jejunum or ileum portions of the small intestine of the subject and substantially reaches the large intestine as well. In a particular embodiment, the enteric coating permits the release of the therapeutically active ingredient from the core once the composition reaches the junction between the duodenum and jejunum portions of the small intestine, such that the release of the therapeutically active ingredient begins at the jejunum portion and occurs until the composition reaches at least the distal small intestine (ileum) as well as the large intestine in effective quantity.

[0106] The enteric coating of an enteric coated composition according to the invention can be used to control the location within the gastrointestinal tract that the glycerophosphate salt is released from the core of the composition, such that the glycerophosphate salt is site-specifically delivered. As used herein, the term “site specific delivery” refers to release of the therapeutically active ingredient from the core of a composition at the intended site of delivery. According to embodiments of the present invention, an enteric coated composition site specifically delivers a glycerophosphate salt to the intestine, preferably the small intestine or large intestine, more preferably the small intestine, and most preferably the junction between the duodenum and jejunum portions of the small intestine. Thus, the enteric coating permits transition of the composition through the stomach of the subject without releasing glycerophosphate salt, and only once the composition passes into the intestinal tract and the enteric coating begins to degrade or dissolve, is the glycerophosphate salt released.

[0107] In a preferred embodiment, once the enteric coating of an enteric coated composition according to the invention has disintegrated or dissolved, substantially all of the glycerophosphate salt is released from the core of the composition at substantially the same time. In a more preferred embodiment, substantially all of the glycerophosphate salt is released from the core of the composition at the time the composition reaches the small intestine of the subject

[0108] In one embodiment, an enteric coated composition according to the invention site-specifically delivers a glycerophosphate salt to the small intestine. When site-specific delivery is to the small intestine, the delivery can be to either the duodenum, jejunum, or ileum portions of the small intestine. In another embodiment, site-specific delivery is to the large intestine.

5 Preferably, an enteric coated composition according to the invention site-specifically delivers a glycerophosphate salt to the junction between the duodenum and jejunum portions, such that maximal release of the glycerophosphate salt does not commence until the composition reaches at least the jejunum, where there is the greatest likelihood that it will not be absorbed into the body like a nutrient, but will proceed into the ileum portion of the small intestine and large intestine to
10 have these sections.

[0109] According to embodiments of the present invention, the enteric coating is comprised of a polymer material. Preferably, the polymer material is pH sensitive, such that the polymer material remains intact, or is stable, at a highly acidic pH, but breaks down, dissolves, or disintegrates at a less acidic pH. A suitable pH sensitive polymer for use with the present
15 invention is one which remains intact and is stable within the lower pH environment of the stomach (i.e., around pH 3), but will disintegrate or dissolve at a pH commonly found in the intestinal tract (i.e., around pH 5 to about pH 7).

[0110] According to embodiments of the present invention, the polymer material can be a synthetic material, such as acrylic or maleic based polymers and polyvinyl derivatives, or an
20 organic material, such as a cellulosic based polymer. Preferably, the polymer material is pH sensitive. Examples of pH-sensitive acrylic polymers that can be used in the present invention include, but are not limited to, polymers (homopolymers or copolymers) of methacrylic acid, methyl methacrylate and ethyl acrylate, etc., such as copoly(methacrylic acid/ethyl acrylate), copoly(methacrylic acid/methyl methacrylate). A copolymer of methacrylic acid and ethyl
25 acrylate is commercially available under the trade name Eudragit® L-30-D 55, available from Evonik Industries. Examples of pH-sensitive cellulosic polymers, that can be used in the present invention include, but are not limited to, hydroxypropylmethylcellulose phthalate (HPMCP), hydroxypropylmethylcellulose acetate succinate (HPMCAS), carboxymethylethyl cellulose (CMEC), cellulose acetate phthalate (CAP), cellulose acetate succinate (CAS), and cellulose
30 acetate trimellitate (CAT). Other commercially available polymers and enteric coating systems that can be used with the present invention include Acryl-EZE®, Sureteric®, Nutrateric® II, and Opadry® enteric coatings all available from Colorcon, Ltd.

[0111] According to embodiments of the present invention, an enteric coating can be comprised of any polymer material, or any combination thereof, in view of the present disclosure. The polymer material can comprise about 5-90% by weight, relative to the total weight of the enteric coating, such as, for example, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%.

5 [0112] According to embodiments of the present invention, an enteric coating is stable in the acidic environment of the stomach, which has a pH of around 3, but is dissolved or degraded in a less acidic environment having a pH of about 5 or greater, such as the pH found in intestinal tract. For example, an enteric coating can be stable at a pH of about 1, 1.5, 2, 2.5, 3, 3.5 or 4, 4.5, or 5 but can be dissolved or disintegrated at a pH of about 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 or 9.

10 [0113] An enteric coating according to the invention can further comprise additives, such as plasticizers, lubricants, pigments, colorants, antifoam agents, emulsifiers, surfactants, etc., to improve the properties of the enteric coating. For example, a plasticizer can increase the flexibility and elasticity, and diminish the brittleness, of the enteric coating. A plasticizer can be either a hydrophilic plasticizer or a hydrophobic plasticizer. Examples of plasticizers that can be
15 used in an enteric coating of the present invention include, but are not limited to, phthalic derivatives, dibutylphthalate, propylene glycol, triacetine, silicone oil, diethylphthalate, triethyl citrate, dibutyl sebacate, and polyethylene glycol, etc. Examples of lubricants that can be used in an enteric coating include magnesium stearate, talc, etc.

20 [0114] In a preferred embodiment, an enteric coating further comprises a plasticizer. The plasticizer can comprise about 5% to about 60% by weight relative to the total weight of the enteric coating, such as about 10%, 20%, 30%, 40%, 50%, or 60%.

[0115] The pH of the intestinal tract gradually increases from a pH of about 5, found in the upper portions of the small intestine such as the duodenum, to a pH of about 7.0-8.0 in the distal portions of the small intestine, such as the ileum, and the large intestine. An enteric coated
25 composition according to the invention is formulated such that the enteric coating will not dissolve and permit release of the glycerophosphate salt until the composition has passed through the stomach and at least reached the upper portion of the small intestine. Thus, the enteric coating can begin to dissolve within the pH range of the duodenum, and continue to dissolve at the pH range within the small intestine. The enteric coating can also be such that it does not
30 begin to dissolve until reaching distal portions of the small intestine (i.e., ileum), or the large intestine.

[0116] Preferably, an enteric coated composition according to the invention permits the quantitative release of the glycerophosphate salt from the core as of the time the composition

reaches at least the small intestine or large intestine of the subject. More preferably, an enteric coated composition according to the invention permits the quantitative release of the glycerophosphate salt from the core as of the time the composition reaches at least the jejunum portion of the small intestine.

5 [0117] The site within the intestinal tract in which the glycerophosphate salt will be released from an enteric coated composition according to the invention can be adjusted by varying several parameters, including the thickness of the enteric coating, identity of the polymers in the enteric coating, pH sensitivity of the enteric coating, addition of additives, etc. Preferably, the enteric coating is such that it is substantially dissolved during the transit time through the intestinal tract to provide for maximum release of the glycerophosphate salt into the intestinal tract, and
10 preferably at the site of the jejunum or ileum.

[0118] In one embodiment, the enteric coating dissolves at a pH of about 5.5 and will site-specifically deliver the glycerophosphate salt to the duodenum portion of the small intestine. In another embodiment, the enteric coating dissolves at a pH between about 5.5 and 7.0, and will
15 site-specifically deliver the glycerophosphate salt in either the jejunum or ileum portion of the intestine. In another embodiment, the enteric coating dissolves at a pH of about 7.0 or greater, and will site specifically-deliver the glycerophosphate salt to the large intestine. Preferably, the enteric coating dissolves at a pH between about 5.5 and 7.0.

[0119] According to embodiments of the present invention, the enteric coating can be present in
20 a weight ratio relative to the core of between about 5% to 60%, and preferably 5% to 30%, such as, for example 10%, 25%, 20%, or 25% (e.g., the enteric coating is 5% by weight relative to total weight of the core). However, one of ordinary skill in the art will recognize that the weight ratio of the enteric coating, and the weight ratio of the composition itself, can be adjusted depending on the intended site of delivery for the glycerophosphate salt. For example, a weight ratio
25 relative to the core of between about 5% and 30% can be appropriate for delivery to the duodenum and jejunum portions of the small intestine, but the weight ratio can be higher than 30% for delivery to the ileum portion of the small intestine, or the large intestine.

[0120] According to embodiments of the present invention, the thickness and/or technical sophistication (i.e. number of layers, type of polymers, etc.) of the enteric coating can affect the
30 rate of dissolution of the enteric coating, and thus the location of subsequent release of the glycerophosphate salt from the core (i.e., the location of site-specific delivery). As used herein, the terms "thin" and "thick" encompass both the thickness of the coating and its technical sophistication. For example, a thin enteric coating will be dissolved or degraded more rapidly

than a thick enteric coating, and release of a glycerophosphate salt from a composition comprising a thin enteric coating can occur at a location in the intestine that is more proximal to the stomach, such as the duodenum, as compared to the location in the intestine that the glycerophosphate salt is released from a composition comprising a thicker enteric coating, which will be more distal to the stomach, such as in the jejunum, ileum, or large intestine.

[0121] The thickness of the enteric coating of an enteric coated composition according to the invention can be controlled by, for example, adjusting the number of layers of the enteric coating. In one embodiment, an enteric coated composition comprises one layer of an enteric coating. In another embodiment, an enteric coated composition comprises more than one layer of an enteric coating, such as for example, 1, 2, 3, 4, or 5 or more layers. For example, increased layers of enteric coating can be used to formulate an enteric coated composition for site-specific delivery to the distal portions of the small intestine, such as the ileum, or the large intestine.

[0122] An enteric coated composition according to embodiments of the present invention can further comprise a subcoat between the enteric coating and the core. As used herein, the term "subcoat" refers to a film or coating that acts as a physical barrier between the core comprising the therapeutically active ingredient and the enteric coating surrounding the core, preventing the enteric coating from being in physical contact with the core. The subcoat is applied as a layer over the core prior to application of the enteric coating. Functions of subcoats include protection of the components of the core, such that there are no adverse reactions between the components of the core and the components of the enteric coating. For example, an enteric layer can be acidic, and direct contact of the core with an acidic enteric layer can result in destabilization or degradation of the therapeutically active component, generation of impurities, etc. A subcoat can also function to provide a smooth base over the core for even application of the enteric coating. Examples of materials suitable for use as a subcoat include, but are not limited to, cellulose polymers, such as methylcellulose, ethylcellulose, etc., acrylics, such as homo or copolymers of methacrylate and methyl methacrylate, etc., and vinyls, such as polyvinyl alcohol, etc. A subcoat can further comprise additives, such as plasticizers, lubricants, pigments, colorants, antifoam agents, emulsifiers, surfactants, etc.

[0123] An enteric coated composition according to the invention is formulated for oral administration. According to embodiments of the present invention, the core of an enteric coated composition is preferably a solid, such as, for example, a tablet, capsule, pellet, liquid gel, granule, powder, etc. Preferably, the core is in the form of a tablet. To prepare the core of an

enteric coated composition, the glycerophosphate salt is mixed with a pharmaceutically acceptable carrier according to conventional pharmaceutical compounding techniques.

[0124] The core can comprise any pharmaceutically acceptable carrier in view of the present disclosure. For example, a pharmaceutically acceptable carrier can include one or more excipients such as binders, suspending agents, emulsifying agents, wetting agents, lubricants, flavorants, sweeteners, preservatives, dyes, and coatings. In particular, suitable carriers and additives for a core formulated as a solid (e.g., tablet, capsule, etc.) include starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents and the like.

[0125] The core of an enteric coated composition for use in the present invention can comprise a therapeutically effective amount of a glycerophosphate salt that is about 10-100%, preferably 50-100%, and most preferably 75-99.9% of the total weight of the core. The amount of glycerophosphate salt will depend on the specific cation of the glycerophosphate salt. For example, if calcium glycerophosphate is used, the core preferably comprises about 99.625% calcium glycerophosphate by weight of the total core, and 0.375% by weight of a carrier(s), and preferably 0.375% by weight magnesium stearate with no other carrier(s), by weight of the total core.

[0126] According to embodiments of the present invention, the core of an enteric coated composition can further comprise an NSAID. The core can comprise an amount of the glycerophosphate salt by weight that is about 10% to about 1000% of the weight of the NSAID in the mixture of glycerophosphate salt and NSAID, preferably 30% to 70%, more preferably 40% to 60%, and most preferably 50% of the weight of the NSAID in the core. In a preferred embodiment, the NSAID is ibuprofen.

[0127] According to embodiments of the present invention, an enteric coated composition of the present invention can further comprise a controlled-release agent in at least one of the core or the enteric coating. As used herein, the term "controlled-release agent" refers to a compound or additive that controls the rate at which the therapeutically active ingredient of a composition is made available, or is tailored to possess a specific delay to govern the site at which the therapeutically active ingredient is made available to the subject to whom the composition is administered. Examples of controlled release agents include sustained release agents (for sustained release or extended release), immediate release agents (for immediate release or dispersion), and delayed release agents (for delayed release).

[0128] As used herein, the terms "sustained-release" and "extended release" refer to the release of the therapeutically active component from a pharmaceutical composition over an extended

period of time. As used herein, the term “immediate release” refers the release or dispersion of the therapeutically active component from a pharmaceutical composition immediately or shortly following administration to a site in a subject. Immediate release can also refer to dispersion directly after the enteric coating of an enteric coated composition according to the invention has begun to dissolve. As used herein, the term “delayed release” refers to the release of the therapeutically active component from a pharmaceutical composition only after the composition reaches a desired locale within the subject following administration to a subject, or a specified amount of time following administration to the subject has past, such that release occurs site-specifically, or at the desired locale.

[0129] Preferably, a composition according to the invention comprises a controlled-release agent that is a delayed-release agent.

[0130] As used herein, the term “delayed-release agent” refers to a compound or additive that provides for the controlled release of the therapeutically active ingredient from a composition, such that release occurs site-specifically, or at the desired locale. A delayed-release agent can be of the type which releases substantially all of the therapeutically active ingredient at the same time, once it reaches the desired locale, or releases the therapeutically active ingredient only after a specified amount of time following administration of the composition to the subject has past, such that the composition reaches a desired locale, and the therapeutically active ingredient is thus delivered to the desired locale, or site-specifically. According to embodiments of the present invention, the delayed-release agent can be present in the composition as part of the core, or as part of the enteric coating. Preferably, the delayed-agent is present as part of the enteric coating.

[0131] According to embodiments of the present invention, an enteric coated composition further comprising a delayed-release agent allows for the release of the glycerophosphate salt from the core only once the composition has at least entered the small intestine or large intestine, and preferably has reached the junction between the duodenum and jejunum portions of the small intestine. Preferably, release of the glycerophosphate salt from the core of the composition can occur over a governing pH range that is at least greater than 5.0, such as 5.5, 6.0, 6.5, 7.0, 7.5, or 8.0 or greater, and preferably occurs at a pH that is at least 7.0 or greater.

[0132] The amount of the delayed-release agent present in a composition according to the invention will depend upon the desired release profile of the glycerophosphate salt. For example, the delayed-release agent can be present in a percentage by weight that is between about 10% and 90% by weight of the total weight of the composition, such as 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%. When the delayed-release agent has a release profile that is particularly pH

durable (i.e., does not dissolve until higher pHs, such as at least pH 7.0), the delayed-release agent can be present in a percentage by weight of the total weight of the composition that can be smaller, such as 10%, 20%, 30%, 40%, or 50%.

[0133] Examples of delayed-release agents suitable for use in the present invention include, but are not limited to, gels, waxes, fats, emulsifiers, combinations of fats and emulsifiers, polymers, starch, cellulose polymers, etc. and combinations thereof. Examples of waxes and waxy materials that can be used to provide delayed-release include canuba wax, spermaceti wax, candellila wax, cocoa butter, cetosteryl alcohol, beeswax, partially hydrogenated vegetable oil, cerasin, paraffin, myristyl alcohol, stearyl alcohol, cetyl alcohol, and stearic acid.

[0134] Preferably, a delayed-release agent comprises a rate-controlling polymer. As used herein, the term "rate-controlling polymer" refers to a polymer that controls the rate at which a therapeutically active ingredient is released from a composition, such that the therapeutically active ingredient is not released until the composition has reached a desired locale, or intended site of delivery. Examples of rate controlling polymers include cellulose polymers, such as hydroxypropylmethylcellulose (HPMC), hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), carboxy methyl cellulose (CMC), and mixtures thereof. According to embodiments of the present invention, the molecular weight of a cellulose polymer to be used as a delayed-release agent can be varied to optimize the rate of controlled-release of the glycerophosphate from the core in order to achieve the desired release profile.

[0135] There are several commercially available rate-controlling polymers suitable for use in the present invention. These include METHOCEL® Cellulose Ethers and ETHOCEL® Ethylcellulose polymers, available from Colorcon, Ltd.

[0136] According to a preferred embodiment of the present invention, a rate-controlling polymer is a pH sensitive polymer that is present in a composition according to the invention as part of the enteric coating. In this embodiment, the enteric coating itself provides delayed-release properties to the composition, such that the enteric coating does not dissolve and allow for release of the glycerophosphate salt from the core until the composition reaches a site of the intestinal tract that has a pH at which the pH sensitive polymer will dissolve, degrade, or disintegrate.

[0137] Methods are known in the art for forming enteric coated compositions, and particularly for forming enteric coated compositions wherein the composition is in the form of a solid for oral administration. An enteric coated composition for use in a method of the present invention can be produced by applying either a solution or suspension of the enteric coating to a core comprising a therapeutically effective amount of a glycerophosphate salt, wherein the core is

preferably in a solid oral dosage form, such as a tablet, capsule, pellet, liquid gel, granule, powder, etc., and is more preferably a tablet.

[0138] According to embodiments of the present invention, a coating formulation for application to the core is first produced. As used herein, the term “coating formulation” refers to an enteric coating according to the invention that is in the form of a suspension/dispersion or a solution. Thus, the coating formulation comprises all the components of the enteric coating either suspended or dissolved in a solvent (organic or aqueous). The coating formulation can be either an aqueous suspension or dispersion, or it can be an organic solution.

[0139] A coating formulation in the form of an aqueous suspension or dispersion can be prepared by diluting or suspending a polymer material and any additives, such as lubricants, plasticizers, delayed-release agents etc., in an aqueous solvent, and preferably in water. A coating formulation in the form of a solution can be prepared by dispersing or dissolving a polymer material and any additives in an organic solvent. Examples of suitable solvents that can be used include acetone, methanol, ethanol, isopropyl alcohol, ethyl acetate, methylene chloride or mixtures thereof, etc. Preferably, a plasticizer is added to the coating formulation, whether it is in the form of an aqueous suspension/dispersion or solution.

[0140] Any method known in the art for forming suspensions and solutions can be used in view of the present disclosure, including, but not limited to, mixing, blending, stirring, etc.

[0141] The coating formulation is then applied to the core to obtain an enteric coated composition according to the invention. Any method known in the art for applying the coating formulation to the core to produce an enteric coated composition according to the invention can be used. Typical coating methods for applying a coating formulation include spray coating methods such as fluidized bed processing, spray drying, and side vented pan coating processes. Examples of machines that can be used to carry out spray coating methods include a fluidized bed coating machine, a centrifugal fluidized bed coating machine, a pan coating machine, and the like. Generally, such processes involve applying a coating formulation, as described above, by spraying the coating formulation onto the core using a spray nozzle, followed by drying to allow evaporation of the solvent originally present in the coating formulation. The temperature of the drying step should be in the range advised by the manufacturer of the particular enteric coating being used.

[0142] According to embodiments of the present invention, a coating formulation is applied to the core at least once, but can be applied to the core more than once to obtain an enteric coated composition with one or more layers of enteric coating. In certain embodiments, it can be

advantageous to apply more than one layer of enteric coating, wherein each layer comprises a different polymer material, or additive component, in order to optimize the dissolution properties, or release profile, of the glycerophosphate salt from the enteric coated composition.

[0143] A subcoat can also be applied to the core prior to application of the enteric coating.

5 Techniques are known in the art for applying subcoats to solid pharmaceutical compositions for oral administration. Such techniques include spraying the subcoat onto particles, such as tablets, capsules, etc., present in a rotating pan, using a fluidized bed coating apparatus, and the like.

[0144] The release profile of the glycerophosphate salt from the core of the enteric coated composition will be affected by a variety of parameters including the thickness of the enteric coating, additives in the coating, the solubility of the glycerophosphate salt, and the pH sensitivity of the polymer material. One of ordinary skill in the art will be able to readily optimize these parameters in order to obtain an enteric coated composition for use in a method of the present invention with the desired dissolution or release profile.

15 Methods of Treatment Using Enteric Coated Compositions

[0145] Enteric coated compositions comprising a therapeutically effective amount of a glycerophosphate salt, preferably calcium glycerophosphate, can be used in any method for treating, reducing, alleviating, or preventing intestinal injury or symptoms thereof in a subject in view of the present disclosure.

20 [0146] According to embodiments of the present invention, the intestinal injury to be treated or prevented can be aggravated by an NSAID (preferably ibuprofen), exercise-induced, or exercise-induced and further aggravated by ingestion of an NSAID. Preferably, the intestinal injury to be treated or prevented is aggravated by an NSAID. For example, enteric coated compositions according to the invention can be used in a method for treating or preventing intestinal injury or symptoms thereof aggravated by an NSAID in a subject, for treating or preventing exercise-induced intestinal injury or symptoms thereof in a subject, or for treating or preventing exercise-induced intestinal injury or symptoms thereof further aggravated by an NSAID in a subject.

25 [0147] Preferably an enteric coated composition is orally administered to a subject. Preferably, the glycerophosphate salt is calcium glycerophosphate.

30 [0148] In a particularly preferred embodiment, the present invention provides a method of treating or preventing intestinal injury or symptoms thereof, including those which are exercise-induced, aggravated by an NSAID in a subject, wherein the subject is ingesting an NSAID, comprising orally administering to the subject an enteric coated, composition according to the

invention, wherein the glycerophosphate salt present in the core of the composition is calcium glycerophosphate. In a preferred embodiment, the NSAID is ibuprofen.

[0149] In another particularly preferred embodiment, the method comprises administering to the subject an enteric coated composition having a core comprising an effective amount of glycerophosphate salt, preferably calcium glycerophosphate, and an effective amount of NSAID, preferably ibuprofen, such that the NSAID and calcium glycerophosphate, are co-administered as part of the same composition.

[0150] In another particularly preferred embodiment, the enteric coated composition further comprises a delayed release agent.

[0151] Enteric coated compositions comprising a therapeutically effective amount of a glycerophosphate salt, preferably calcium glycerophosphate, can be used in any method for increasing tight junction integrity in a subject in need thereof and for treating or preventing a disease related to tight junction dysfunction as previously described in a subject in need thereof in view of the present disclosure.

[0152] It is believed that the functioning moiety of the glycerophosphate salt is the glycerophosphate component. Although calcium is the preferred cation for the glycerophosphate salt, nonetheless, the presence of calcium along with the glycerophosphate component is not essential to the activity of glycerophosphate in this arena and other appropriate non-toxic cations can be used in the present invention including, but not limited to, glycerophosphate salts of sodium, potassium, and magnesium. However, CGP is the preferred glycerophosphate salt because a loosely bound calcium ion is present in CGP which breaks away from the glycerophosphate moiety immediately upon encountering moisture, resulting in the instantaneous availability of both ions in an aqueous milieu, and which, while present in minimal quantities relative to already existing calcium, still is implicated in its reinforcement of the effect of glycerophosphate on the engendering and longevity of SIP. Further, to the degree that the normal E-cadherins are functioning, calcium in any amount is beneficial; the very name "cadherin" is derived from "calcium-dependent adhesion." It should be emphasized, however, that the CGP effect is not the consequence of added calcium, since the media already contains 1mM calcium, and there are effects demonstrated at 1 μ M GCP.

[0153] The use of CGP and other glycerophosphate salts for additional purposes not disclosed in the present application has been described in various other patents, both pending and granted. For example, U.S. Patent No. 5, 869,119 describes the use of CGP in increasing the pH of acidic foods and beverages to reduce heartburn and gastrointestinal distress, and U.S. Patent No.

7,402,323 describes the beneficial effects of CGP in cellular repair. While not wishing to be bound by theory, it is believed that the beneficial effects of CGP in various applications are achieved synergistically between the calcium ion and the glycerophosphate moiety present in the CGP molecule. In preventing and/or treating exercise-induced intestinal injury and exercise-induced intestinal injury aggravated by ingestion of an NSAID according to the present invention, CGP may be instrumental in, among other benefits, fomenting tight junctions among intestinal cells. For this reason, CGP is effective at increasing tight junction integrity and preventing or treating diseases related to tight junction dysfunction.

[0154] This invention will be better understood by reference to the non-limiting examples that follow, but those skilled in the art will readily appreciate that the examples are only illustrative of the invention as described more fully in the claims which follow thereafter.

EXAMPLES

[0155] Experiments were conducted on Caco-2 cells, a line of heterogeneous human epithelial colorectal adenocarcinoma cells derived from a colorectal carcinoma and grown on transwell permeable inserts. Despite their origins, in culture these cells express the characteristics of small intestinal enterocytes upon prolonged culture (C. Jumarie et al. *J. Cell. Physiol.* 149: 24-33, (1991)). They are widely used as a model of transepithelial transport in the small intestine, and are considered a model for celiac disease (G. Iacomino et al. *J Agric Food Chem.* 6;61(5):1088-962013 (2013); I. Caputo et al. *PLoS One.* 7(9):e45209 (2012); T. Rauhavirta et al. *J Clin Immunol.*; 33(1):134-42 (2013)).

[0156] (1) *Measurement of transepithelial electrical resistance*: The tightness of the tight junction in a transporting epithelium can be evaluated by measuring the electrical resistance across the membrane or trans-epithelial electrical resistance (TEER). TEER measures the movement of ions across the cell (transcellular) and between cells (paracellular). In a very tight monolayer, transport is nearly all transcellular, hence the electrical resistance is high. Cells are grown on a permeable barrier (e.g., the transwell system), and the electrical resistance across the membrane is measured using an ohmmeter (e.g., the EVOM2 epithelial volttohmmeter) specifically designed for the purpose. At 3 to 4 weeks in culture, the TEER may be in the range of 700-800 Ω/cm^2 .

[0157] (2) *Mannitol flux*: The TEER results are verified by measuring the flux of mannitol, a polyhydroxy alcohol, across the cell monolayer. Mannitol flux is exclusively by the paracellular route, so that its movement is inversely proportional to the tightness of the tight junctions. Flux is measured in both apical to basolateral and basolateral to apical directions. The rate of flux is

used to calculate a permeability coefficient for the monolayers. The permeability coefficient for mannitol may be inversely related to TEER.

[0158] (3) *E-cadherin expression*: E-cadherin is the principle protein of tight junctions. The level of E-cadherin is measured in homogenized Caco-2 cells by western blot analysis using commercially available antibodies. Since it is possible that E-cadherin might be expressed as a protein, but sequestered away from the cell membrane, surface E-cadherin is also measured using either flow cytometry or fluorescent microscopy. The ratio of surface to total E-cadherin is calculated. The expression levels of E-cadherin are measured in Caco-2 cells in the presence and absence of CGP. The expression levels of other proteins of tight junctions in Caco-2 cells in the presence or absence of CGP can also be measured using methods known in the art in view of the present disclosure.

[0159] (4) *Sphingosine-1-Phosphate*: Calcium glycerophosphate has activity as a phosphatase inhibitor. It is believed that CGP elevates S1P concentrations by inhibiting dephosphorylation. S1P concentrations are measured in the media of Caco-2 cells using a commercially available ELISA kit (e.g., Echelon Catalog # K-1900). The SIP concentrations are measured in the media of Caco-2 cells in the presence and absence of CGP.

Experimental Conditions

[0160] *Baseline values* were obtained for each of these parameters in unstimulated cells in the presence and absence of increasing concentrations of CGP. The time course of TEER development, as well as the appropriate concentration of CGP, was determined experimentally. The TEER development in Caco-2 cells takes from 18 to 24 days after the monolayers reach confluence, while the CGP concentrations are anticipated to range from 1 μ M to 1 mM. The mannitol flux and TEER data are used to differentiate transcellular and paracellular permeabilities. At baseline, paracellular permeability can be very low relative to transcellular permeability, reflecting the “tightness” of the tight junctions.

[0161] *The effect of hypoxia* on transepithelial permeability was measured for each parameter in the presence and absence of CGP. Cells were grown to confluence in normoxic conditions on transwell inserts, then transferred to a hypoxia chamber (95% N₂/5% CO₂) and media was changed to one sparged with 95% N₂/5% CO₂ and containing no glucose. Under these conditions, which simulate ischemia, the partial pressure of residual oxygen is less than 25 mm Hg (6%). Control wells contained only media; the remaining wells contained increasing concentrations of calcium glycerophosphate. TEER and mannitol flux were measured in the

hypoxia chamber. E-Cadherin expression or S1P determination was determined in room air using samples prepared in the hypoxia chamber. Hypoxia may decrease TEER and S1P, increase both apical to basolateral and basolateral to apical mannitol flux without changing E-cadherin expression. These effects may be mitigated by CGP.

[0162] *The effect of exogenous cytokines* was measured for each parameter in the presence and absence of CGP. Both hypoxia and celiac disease are characterized by the release of cytokines by cells of the immune system. These inflammatory mediators increase monolayer permeability (M. Bayardo et al. *Clin Exp Immunol.*;168(1):95-104 (2012)). Cells were grown to confluence on transwell inserts, then challenged with a mixture of the cytokines TNF α (tumor necrosis factor alpha), IL β (interleukin beta), and IF γ (interferon gamma) ("cytomix") in the presence or absence of increasing concentrations of CGP. At the appropriate time points, apical to basolateral mannitol flux was measured. TEER, E-Cadherin expression and media concentrations of S1P were measured in separate experiments. In addition, the expression of iNOS was measured to verify that the cytokine treatment resulted in an inflammatory response. Exogenous cytokines may decrease TEER and increase mannitol flux.

[0163] *The effect of α -gliadin peptides* is measured for each parameter. α -Gliadin is the offending protein initiating the symptoms of celiac disease in susceptible patients. Gastric and pancreatic proteases cleave α -gliadin to peptides, which may be the direct cause of the enteropathy. The literature (G. Iacomino et al. *J Agric Food Chem.* 6;61(5):1088-962013 (2013)) suggests that the peptide comprised of α -gliadin amino acids 31-55 is a likely candidate. A most likely candidate (consensus of other investigators) was selected and each parameter in these experimental conditions was measured, as shown in Table 1:

TABLE 1

Peptide 31-55	CGP	Cytokine
Present	Absent	Absent
Present	Present	Absent
Present	Present	Present

[0164] The peptides may increase permeability, and may act synergistically with the cytokines. CGP may return permeability toward normal.

[0165] *Mannitol Flux Assay* was performed as follows. At the start of the experiment (time 0), the media on the cells was changed to one containing no glucose and no fetal calf serum, and the environment was changed from 95% oxygen/5% CO₂, to 1% oxygen, 5% CO₂ and 94% nitrogen. These conditions mimic ischemia (no food, no oxygen).

Experimental Results and Discussion

[0166] *Baseline: Mannitol Flux.* The effect of CGP on baseline mannitol flux measured in Caco-2 cells grown on transwell permeable inserts is shown in Fig. 1. There was no significant effect of CGP on mannitol flux across the barrier. However, it is noted that at each of the time points, the mannitol permeability was greatest in the untreated cells.

[0167] *Baseline: TEER.* The effect of CGP on baseline TEER is shown in Fig. 2. It may be seen that citrate produces a small but statistically significant reduction in TEER, particularly noticeable at two hours. This is prevented by CGP, although the CGP effect is gone by four hours.

[0168] *Baseline: SIP.* The effect of CGP on sphingosine 1-phosphate concentrations in the media is shown in Fig. 3. Cells were incubated for 24 hours in the presence of increasing concentrations of CGP. The media was removed and assayed for CGP by enzyme-linked immunoassay (ELISA). It may be seen that under baseline conditions, CGP does not appear to have any effect on sphingosine 1-phosphate concentrations.

[0169] *Baseline: E-Cadherin.* It was observed that CGP may increase, but most likely does not change, E-Cadherin expression.

[0170] Altogether, the data show that CGP has only a small effect on transepithelial permeability under baseline conditions.

[0171] *Mannitol Flux Assay.* It was observed that cells responded to the ischemia conditions by letting go of non-essential functions, like maintaining tight junctions. It appears that by adding CGP, the cells were able to retain tight junction integrity for a longer period of time.

[0172] Specifically, Table 2 and Fig. 4 show the percentage increase in mannitol penetration at two and four hours after CGP administration. The movement of mannitol is inversely proportional to the tightness of the tight junctions. It can be seen that the magnitude of the increase in tightness of the tight junctions (or decrease in mannitol flux) was proportional to the concentration of CGP in the media. While it is possible that the cells are merely using the

glycerol phosphate to replace glucose, this is extremely unlikely, as the effect persists even at 0.000001 molar CGP. The usual glucose concentration in the media is around 10 mM, ten times greater than the highest CGP concentration, and 10,000 times greater than the lowest concentration of CGP. Thus, the results in Table 2 and Fig. 4 indicate that administering CGP to the cell resulted in increased tight junction integrity for a longer period of time.

TABLE 2

CGP Concentration (M)	Disintegrations Per Minute	
	2 hours	4 hours
0	188.6054	2362.6050
0.001	23.10722	150.2845
0.0001	50.46854	188.7996
0.00001	67.95316	195.9237
0.000001	77.03592	275.0632

Transepithelial Permeability under Conditions of Hypoxia

[0173] Hypoxia/ischemia is a state of diminished oxygen and nutrient supply such that cellular demands are not met. It was previously observed that heavy exercise, such as long-distance cycling, induces a state of increased transepithelial permeability that is exacerbated by non-steroidal anti-inflammatory agents such as ibuprofen. It was hypothesized that the increased transepithelial permeability is the consequence of intestinal hypoxia (itself a consequence of diversion of blood flow from the gut during heavy exercise), and that the permeability might be alleviated by CGP treatment. The hypothesis was tested by placing Caco-2 cells in a hypoxia chamber and measuring both apical to basolateral mannitol flux and TEER.

[0174] *Mannitol Flux.* Fig. 5 shows the effect of CGP on mannitol flux during hypoxia. For these studies, [^{14}C]mannitol was added to the apical chamber of the transwell, and sampling was from the basolateral chamber. CGP significantly reduced hypoxia induced mannitol flux in a concentration dependent manner. The effect is time dependent, as it was diminished by four hours and absent by five hours.

[0175] *TEER.* Fig. 6 shows the effect of CGP on TEER during hypoxia. For these studies, cells were grown on transwells until confluent, as measured by TEER values of $>600 \text{ m}\Omega/\text{cm}^2$. As

there is some variability in baseline TEER, all data have been expressed as a % of baseline TEER. After 1 hour hypoxia, there was a significant decrease in TEER in the control cells. The control TEER continued to drop for the duration of the experiment. However, CGP preserved TEER in a concentration dependent manner. As for mannitol, the effect of CGP is diminished with time, so that by five hours there was no longer a discernible effect.

[0176] *S1P and E-Cadherin*. (Theoretical Example) It is hypothesized that the effect of CGP is mediated through S1P. The hypothesis is tested by placing cells in the hypoxia chamber in the presence or absence of CGP. At two and four hours, samples of the media are removed and assayed for S-1P and for E-cadherin (the major tight junction protein). It is expected that S-1P will be greater in the CGP treated cells, and that there will be no change in the E-cadherin. These results will indicate that the preservation of transepithelial integrity is mediated via S1P, rather than via E-cadherin.

Transepithelial Permeability Following Cytokine Stimulation

[0177] Cytokines are a category of small proteins that are important in cell-cell signaling. There are many types and functions of cytokines. Three of the cytokines, TNF α (tumor necrosis factor alpha), IL β (interleukin beta), and IF γ (interferon gamma) are involved in cell signaling during inflammation. Hence, treating cells with a combination of TNF- α , IF- γ , and IL- β mimics the early phases of the inflammatory response. Inflammation is relevant to the present study for two reasons. First, neutrophils and other cells mediating the immune response are recruited to ischemic tissue. Second, the intestinal damage of celiac disease is dependent on inflammation. It was hypothesized that cytokine stimulation will increase transepithelial permeability in Caco-2 cells, and that the effect will be reduced by concurrent treatment with CGP.

[0178] *Mannitol Flux*. As shown in Fig. 7, cytomix alone increased transepithelial mannitol permeability in a time-dependent fashion, and CGP delayed the permeability increase in a concentration-dependent manner. Interestingly, in these experiments, control mannitol permeability was significantly greater than in the CGP/cytomix treated cells. This is in contrast to the baseline studies, in which CGP alone appeared to have very little, if any, influence on mannitol flux.

[0179] *TEER*. The results of the cytomix/TEER experiment were quite different and unexpected. As shown in Fig. 8, it is clear that cytomix does not begin to reduce TEER until eight hours exposure. At ten hours, all CGP treated groups except 1 μ M has a TEER

significantly greater than cytomix alone and not different from control. By 24 hours, all CGP effect was lost. These results are in marked contrast to mannitol flux, and may suggest different molecular mechanisms for mannitol flux and TEER maintenance. The results indicate that additional experiments at time points between eight and ten hours would be helpful.

5 [0180] *SIP and E-Cadherin*. (Theoretical Example) It is hypothesized that the effect of CGP is mediated through S-1P. The hypothesis is tested by treating cells with cytomix in the presence or absence of CGP. At 2 hours and 4 hours, samples of the media are removed and assayed for S-1P and for E-cadherin (the major tight junction protein).

[0181] *Effect of α -gliadin peptides (Theoretical Example)*

10 [0182] The α -gliadin peptide fragment 31-55 is the offending peptide initiating the symptoms of celiac disease in susceptible patients. Mannitol flux and TEER are measured for the following experimental settings:

1. Peptide only
2. Peptide plus 1 mM CGP
- 15 3. Peptide plus cytomix
4. Peptide plus cytomix plus 1 mM CGP.

[0183] At the end of the TEER experiments, the media is assayed for S-1P and E-cadherin and the cells themselves are processed for E-cadherin.

20 [0184] It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the appended claims.

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CLAIMS

We claim:

5 1. A method of treating or preventing an intestinal injury or symptom thereof in a subject, the method comprising administering a composition comprising a therapeutically effective amount of a glycerophosphate salt to the subject such that the intestinal injury or symptom thereof is treated or prevented.

2. The method of claim 1, wherein the glycerophosphate salt is calcium glycerophosphate.

10 3. The method of claim 1, wherein the intestinal injury or symptom thereof is exercise-induced, and wherein the subject engages in exercise.

4. The method of claim 1, wherein the composition is an enteric-coated composition comprising:

15 (i) a core comprising the therapeutically effective amount of the glycerophosphate salt; and

(ii) an enteric coating surrounding the core.

20 5. The method of claim 4, wherein the enteric coating prevents release of the glycerophosphate salt from the core until the composition has reached at least the small intestine or large intestine of the subject, such that the intestinal injury or symptom thereof is treated or prevented.

6. The method of claim 4, wherein the intestinal injury or symptom thereof is exercise-induced, and wherein the subject engages in exercise.

7. The method of claim 4, wherein at least one of the core and the enteric coating of the composition further comprises a delayed-release agent.

25 8. The method of claim 4, wherein the core further comprises a nonsteroidal anti-inflammatory drug (NSAID).

9. The method of claim 1, wherein the composition is administered orally or nasally.

10. A method of treating or preventing an intestinal injury or symptom thereof aggravated by a nonsteroidal anti-inflammatory drug (NSAID) in a subject, wherein the subject is administered the NSAID, the method comprising administering a composition comprising a therapeutically effective amount of a glycerophosphate salt to the subject such that the intestinal injury or symptom thereof aggravated by the NSAID is treated or prevented.

11. The method of claim 10, wherein the glycerophosphate salt is calcium glycerophosphate.

12. The method of claim 10, wherein the composition comprises the therapeutically effective amount of the glycerophosphate salt and the NSAID.

13. The method of claim 10, wherein the intestinal injury or symptom thereof is exercise-induced, and wherein the subject engages in exercise.

14. The method of claim 10, wherein the composition is an enteric-coated composition comprising:

(i) a core comprising the therapeutically effective amount of the glycerophosphate salt; and

(ii) an enteric coating surrounding the core.

15. The method of claim 14, wherein the enteric coating prevents release of the glycerophosphate salt from the core until the composition has reached at least the small intestine or large intestine of the subject, such that the intestinal injury or symptom thereof is treated or prevented.

16. The method of claim 14, wherein the intestinal injury or symptom thereof is exercise-induced, and wherein the subject engages in exercise.

17. The method of claim 14, wherein at least one of the core and the enteric coating of the composition further comprises a delayed-release agent.

18. The method of claim 14, wherein the core further comprises a nonsteroidal anti-inflammatory drug (NSAID).

19. The method of claim 10, wherein the composition is administered orally or nasally.

20. An enteric coated composition comprising:

- 5 (i) a core comprising a therapeutically effective amount of a glycerophosphate salt; and
(ii) an enteric coating surrounding the core.

21. The composition according to claim 20, wherein the glycerophosphate salt is calcium glycerophosphate.

10 22. The composition of claim 20, wherein the enteric coating prevents release of the glycerophosphate salt from the core until the composition has reached at least the small intestine or large intestine of the subject.

23. The composition according to claim 20, wherein the core further comprises a non-steroidal anti-inflammatory drug (NSAID).

15 24. The composition according to claim 20, wherein at least one of the core and the enteric coating further comprises a delayed-release agent.

25. A method of increasing tight junction integrity in a subject in need thereof, comprising administering to the subject a composition comprising an effective amount of glycerophosphate.

20 26. The method of claim 25, wherein the composition is an enteric-coated composition comprising:

- (i) a core comprising the therapeutically effective amount of the glycerophosphate salt;
and
(ii) an enteric coating surrounding the core.

27. A method of treating or preventing a disease related to tight junction dysfunction in a subject in need thereof, comprising administering to the subject a composition comprising an effective amount of glycerophosphate.

28. The method of claim 27, wherein the disease is selected from the group consisting of an inflammatory disease and a cancer.

29. The method of claim 28, wherein the inflammatory disease is selected from the group consisting of inflammatory bowel disease (IBD), Crohn's disease, celiac, sprue, ulcerative colitis, multiple sclerosis, a hereditary disease, vision loss, a viral infection, bacterial toxins, neuroinflammation, a disease in lung tissue integrity, a disease in lymphocyte trafficking, and another immunological disease.

30. The method of claim 28, wherein the glycerophosphate is calcium glycerophosphate.

31. The method of claim 28, wherein the composition is an enteric-coated composition comprising:

- (i) a core comprising the therapeutically effective amount of the glycerophosphate salt;
- and
- (ii) an enteric coating surrounding the core.

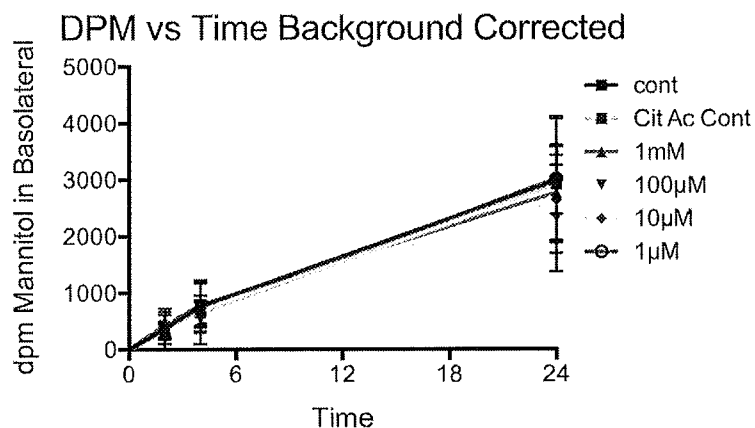


Fig. 1. Baseline mannitol flux in the presence and absence of increasing concentrations of CGP

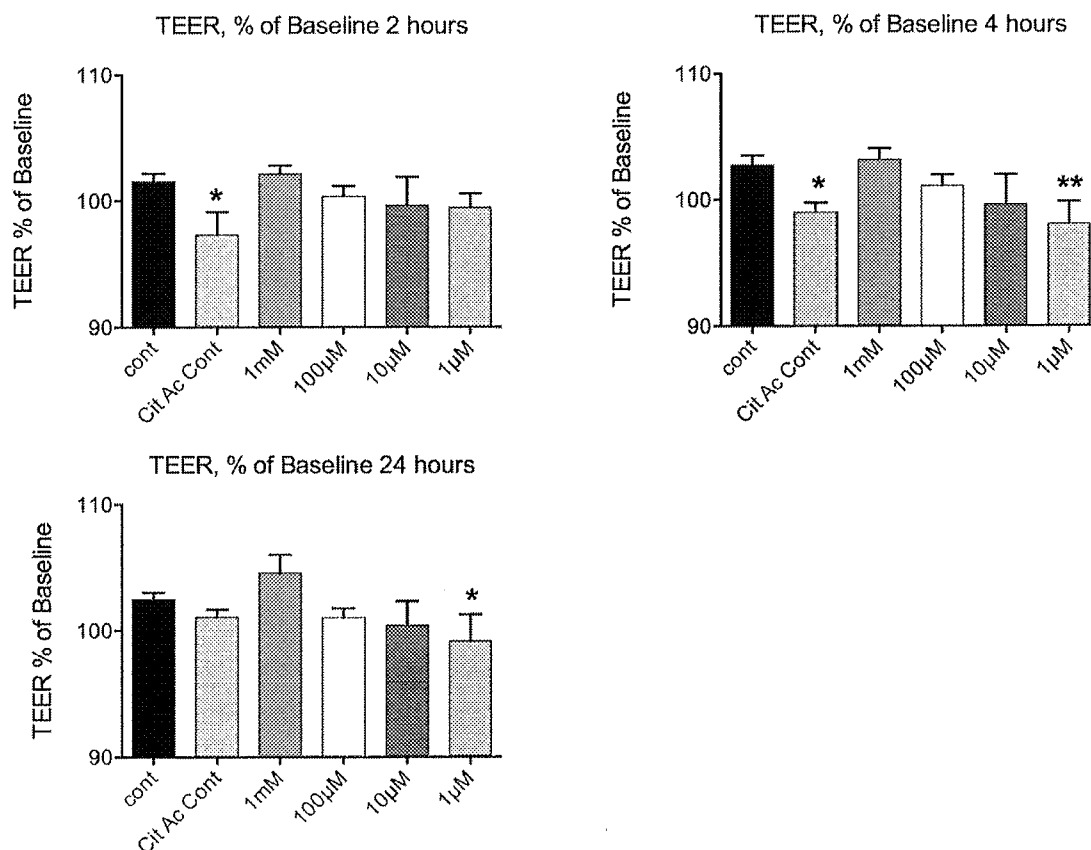


Fig. 2. The effect of CGP on trans-epithelial electrical resistance (TEER) in the presence and absence of increasing concentrations of CGP.

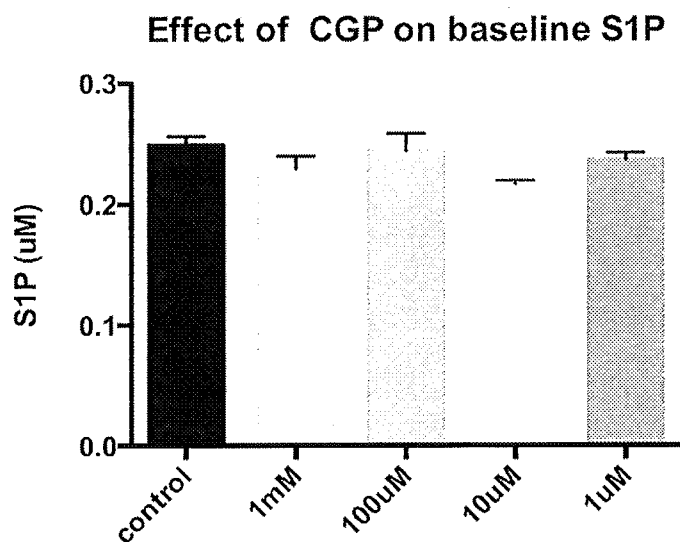


Fig. 3. The effect of CGP on baseline production of sphingosine 1-phosphate in Caco-2 cells.

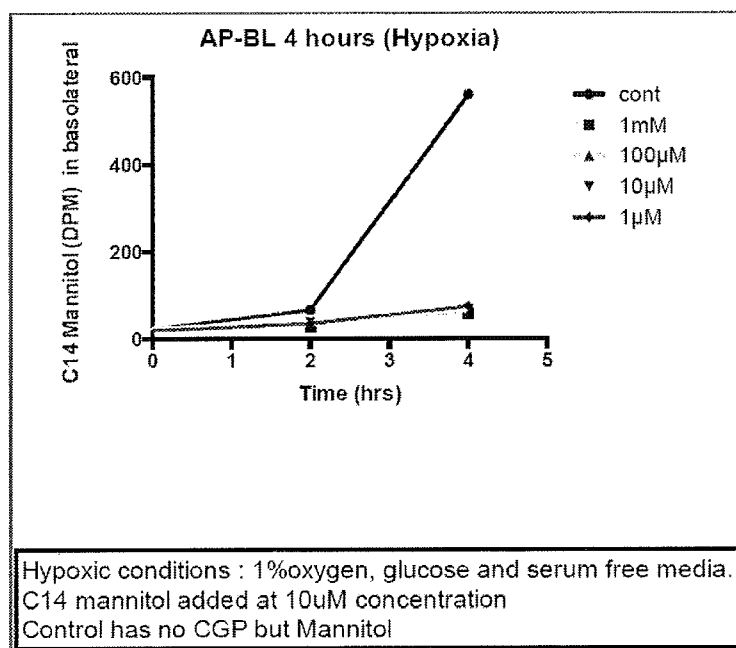


Fig. 4. Increase in mannitol penetration in Caco-2 cells 4 hours after the administration of calcium glycerophosphate (CGP) to the cells.

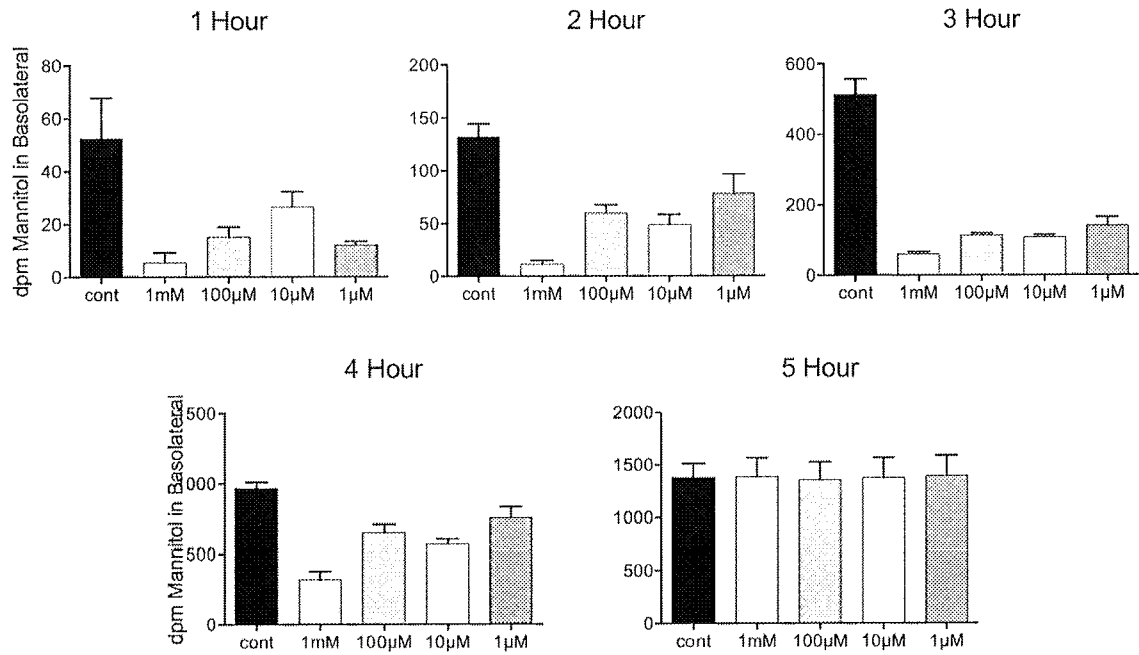


Fig. 5. Transepithelial mannitol flux during hypoxia

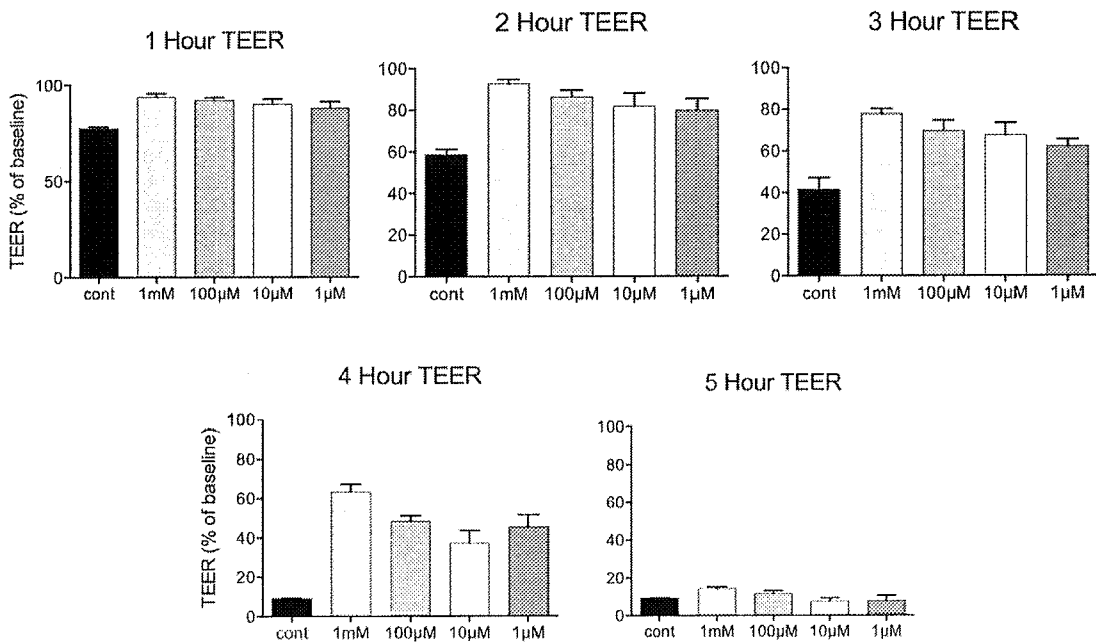


Fig. 6. TEER during hypoxia.

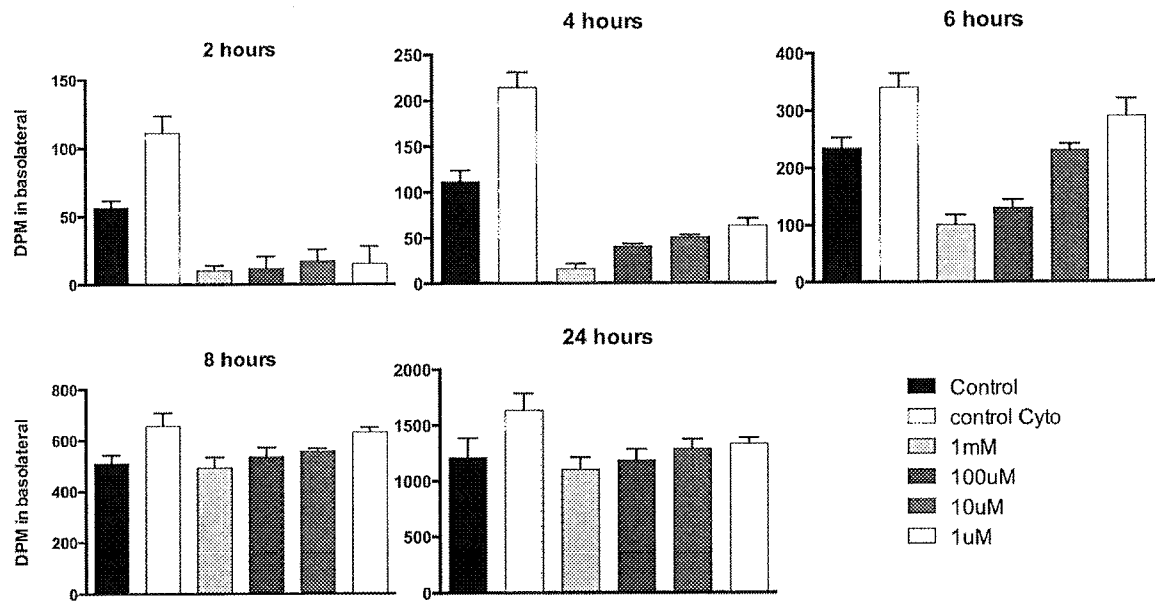


Figure 7. The effect of CGP on transepithelial mannitol flux induced by cytomix.

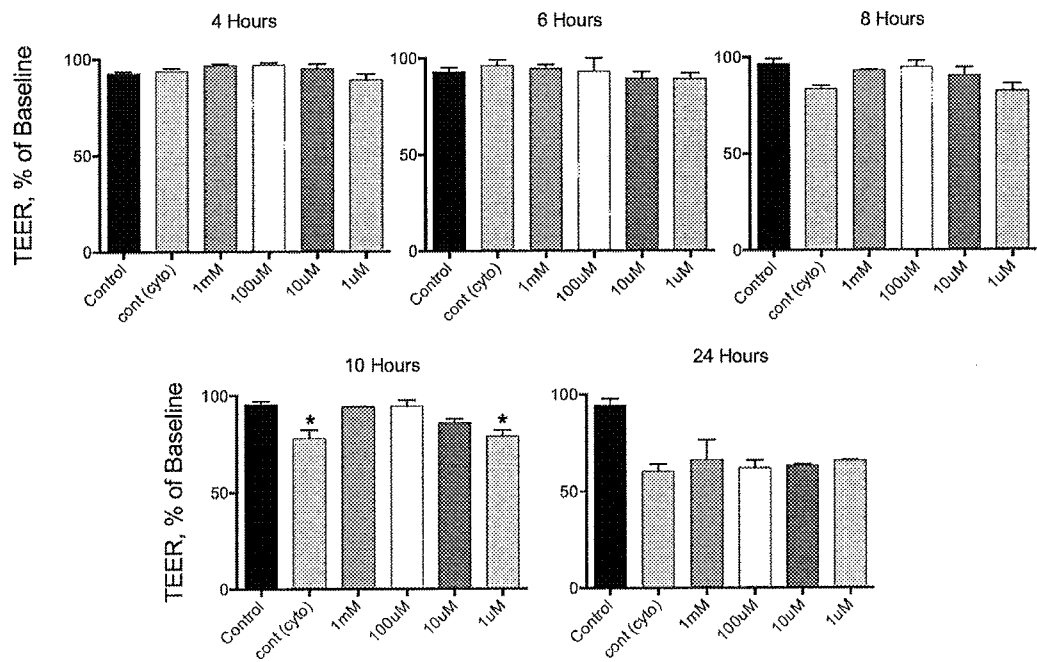


Fig. 8. The effect of CGP on transepithelial electrical resistance during cytomix treatment.



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- (72) **Inventors:** KLIGERMAN, Alan, E.; 3408 Bargaintown Road, Egg Harbor Township, NJ 08234 (US). WEIS, Margaret, T.; Texas Tech University Health, Sciences Center, 1300 Coulter Drive, Amarillo, TX 79106 (US).
- (74) **Agents:** HSING, Weihong et al.; Panitch Schwarze Belisario & Nadel LLP, One Commerce Square, 2005 Market Street, Suite 2200, Philadelphia, PA 19103 (US).
- (81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

- (84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

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- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

[Continued on next page]

- (54) **Title:** METHODS AND COMPOSITIONS OF TREATING AND PREVENTING INTESTINAL INJURY AND DISEASES RELATED TO TIGHT JUNCTION DYSFUNCTION

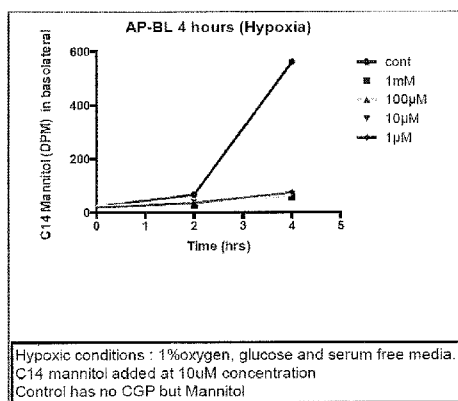


Fig. 4. Increase in mannitol penetration in Caco-2 cells 4 hours after the administration of calcium glycerophosphate (CGP) to the cells.

- (57) **Abstract:** Methods and compositions for treating and preventing intestinal injury are described. In particular, methods and delayed-release enteric coated compositions of glycerophosphate salts for treating and preventing an intestinal injury, such as exercise-induced intestinal injury, intestinal injury induced or aggravated by ingestion of a nonsteroidal anti-inflammatory drug (NSAID), or exercise-induced intestinal injury aggravated by an NSAID, are described. Orally or nasally administering a therapeutically effective amount of a glycerophosphate salt, particularly calcium glycerophosphate, to a subject engaging in exercise, particularly a subject engaging in exercise and ingesting a NSAID for the treatment or prevention of musculoskeletal pain resulting from exercise, reduces exercise-induced intestinal injury, particularly exercise-induced intestinal injury aggravated by the NSAID. A method of increasing tight junction integrity in a subject in need thereof is also described. The method involves administering to the subject a composition comprising an effective amount of glycerophosphate, such as calcium glycerophosphate. A method of treating or preventing a disease related to tight junction dysfunction, such as an inflammatory disease or a cancer, is also described.



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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/017408

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/66 A61P1/00
ADD.

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

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

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

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GOSS SANDRA L ET AL: "Determination of calcium salt solubility with changes in pH and P-CO ₂ , simulating varying gastrointestinal environments", JOURNAL OF PHARMACY AND PHARMACOLOGY, vol. 59, no. 11, November 2007 (2007-11), pages 1485-1492, XP002723976, ISSN: 0022-3573 abstract table 1 page 1490, column 2, paragraph 1 -----	1-19
X	US 2002/099037 A1 (KLIGERMAN ALAN E [US] ET AL) 25 July 2002 (2002-07-25) abstract	1-19
Y	example 1 paragraph [0047] - paragraph [0052] ----- -/-	20-24



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

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Authorized officer

Uryga-Polowy, V

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/017408

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"Prelief tablets and granulate", 13 January 2011 (2011-01-13), XP002723977, Retrieved from the Internet: URL:http://wayback.archive.org/web/2011011 3165631/http://www.drugs.com/drug/prelief-t ablets-and-granulate.html [retrieved on 2014-05-06] the whole document -----	1-19
X	WO 2005/002549 A1 (NYCOMED DANMARK APS [DK]; BOENLOEKKE LISBETH [DK]; CHRISTENSEN KARIN L) 13 January 2005 (2005-01-13)	20-22
Y	claims 3, 4, 11, 13, 20 -----	20-24
A	WO 2005/009381 A2 (UNIV MISSOURI [US]; SANTARUS INC [US]; PHILLIPS JEFFREY O [US]; WIDDER) 3 February 2005 (2005-02-03) claims 1, 11, 12 -----	20-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/017408

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.:
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:

see additional sheet

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 an 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.:
1-24
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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-19

a method of treating or preventing an intestinal injury or symptoms thereof by administering a glycerophosphate salt

2. claims: 20-24

an enteric coated composition comprising (i) a core comprising a glycerophosphate salt and (ii) an enteric coating surrounding the core

3. claims: 25-31

a method of increasing tight junction integrity, of treating or preventing a disease related to tight junction dysfunction by administering a glycerophosphate salt

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2014/017408

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2002099037 A1	25-07-2002	AU 2002236802 B2 CA 2434544 A1 CN 1487834 A EP 1353678 A2 MX PA03006466 A US 2002099037 A1 US 2005130938 A1 WO 02056834 A2 ZA 200305089 A	11-08-2005 25-07-2002 07-04-2004 22-10-2003 15-10-2004 25-07-2002 16-06-2005 25-07-2002 30-06-2004
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代理人 崔佳佳 马莉华

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W02014/130678 EN 2014. 08. 28

(71) 申请人 普瑞丽芙有限公司

地址 美国新泽西州

(72) 发明人 A·E·克利杰曼 M·T·韦斯

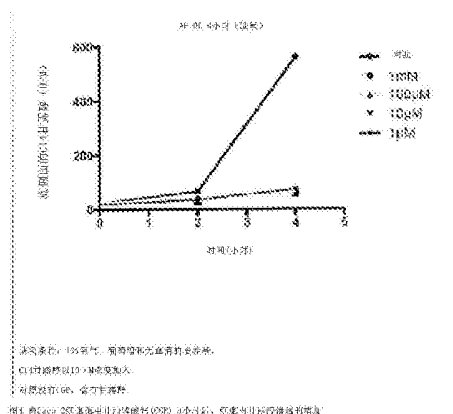
权利要求书2页 说明书22页 附图4页

(54) 发明名称

治疗和防治肠道损伤、紧密连接部功能障碍相关疾病的方法和组合物

(57) 摘要

描述了治疗和预防肠损伤的方法和组合物。具体描述了用于治疗 and 预防肠损伤的方法和甘油磷酸盐的缓释肠溶包衣组合物,所述肠损伤是例如运动诱发肠损伤、由摄入非固醇类抗炎药物(NSAID)诱发或加重的肠损伤、NSAID加重的运动诱发肠损伤。经口或鼻向参与运动的个体,尤其是参与运动并为治疗或预防运动引起的肌肉骨骼痛而摄入NSAID的个体施用治疗有效量的甘油磷酸盐,尤其是甘油磷酸钙以减轻运动诱发肠损伤、尤其是NSAID加重的运动诱发肠损伤。还描述了增强有此需要的个体中紧密连接部完整性的方法。该方法包括向个体施用含有有效量的甘油磷酸盐,比如甘油磷酸钙的组合物。还描述了治疗或预防紧密连接部功能障碍相关疾病,比如炎症疾病或癌症的方法。



1. 一种治疗或预防个体中肠道损伤或其症状的方法,其特征在于,所述方法包括:向所述个体施用含有治疗有效量的甘油磷酸盐的组合物,从而治疗或预防肠道损伤或其症状。

2. 如权利要求 1 所述的方法,其特征在于,所述甘油磷酸盐是甘油磷酸钙。

3. 如权利要求 1 所述的方法,其特征在于,所述肠道损伤或其症状是运动诱发的,并且其中所述个体参与运动。

4. 如权利要求 1 所述的方法,其特征在于,所述组合物是肠溶包衣组合物,包括:

(i) 核心,包括治疗有效量的甘油磷酸盐;和

(ii) 围绕核心的肠溶包衣。

5. 如权利要求 4 所述的方法,其特征在于,直到所述组合物至少到达该个体的小肠或大肠,肠溶包衣才会使得甘油磷酸盐从核心中释放出来,从而治疗或预防肠道损伤或其症状。

6. 如权利要求 4 所述的方法,其特征在于,所述肠道损伤或其症状是运动诱发的,并且其中所述个体参与运动。

7. 如权利要求 4 所述的方法,其特征在于,所述组合物的核心和肠溶包衣的至少一个还含有缓释剂。

8. 如权利要求 4 所述的方法,其特征在于,所述核心进一步含有非类固醇抗炎药物(NSAID)。

9. 如权利要求 1 所述的方法,其特征在于,所述组合物经口或鼻腔给药。

10. 一种治疗或预防个体中由非类固醇抗炎药物(NSAID)加重的肠道损伤或其症状的方法,其特征在于,所述个体服用 NSAID,所述方法包括向该个体施用含有治疗有效量的甘油磷酸盐的组合物,从而治疗或预防由 NSAID 加重的肠道损伤或其症状。

11. 如权利要求 10 所述的方法,其特征在于,所述甘油磷酸盐是甘油磷酸钙。

12. 如权利要求 10 所述的方法,其特征在于,所述组合物含有治疗有效量的甘油磷酸盐和 NSAID。

13. 如权利要求 10 所述的方法,其特征在于,所述肠道损伤或其症状是运动诱发的,并且其中所述个体参与运动。

14. 如权利要求 10 所述的方法,其特征在于,所述组合物是肠溶包衣组合物,包括:

(i) 核心,包括治疗有效量的甘油磷酸盐;和

(ii) 围绕核心的肠溶包衣。

15. 如权利要求 14 所述的方法,其特征在于,直到所述组合物至少到达该个体的小肠或大肠时,肠溶包衣才会使得甘油磷酸盐从核心中释放出来,从而治疗或预防肠道损伤或其症状。

16. 如权利要求 14 所述的方法,其特征在于,所述肠损伤或其症状由运动诱发,并且所述个体参与了运动。

17. 如权利要求 14 所述的方法,其特征在于,所述组合物的核心和肠溶包衣的至少一个还含有缓释剂。

18. 如权利要求 14 所述的方法,其特征在于,所述核心还含有非固醇类抗炎药物(NSAID)。

19. 如权利要求 10 所述的方法, 其特征在于, 所述组合物经口或鼻腔给药。
20. 一种肠溶包衣组合物, 包括:
 - (i) 核心, 所述核心包括治疗有效量的甘油磷酸盐 ; 和
 - (ii) 围绕核心的肠溶包衣。
21. 如权利要求 20 所述的组合物, 其特征在于, 所述甘油磷酸盐为甘油磷酸钙。
22. 如权利要求 20 所述的组合物, 其特征在于, 直到所述组合物至少到达所述个体的小肠或大肠时, 肠溶衣才会使得甘油磷酸盐从核心中释放出来。
23. 如权利要求 20 所述的组合物, 其特征在于, 所述核心还包括非类固醇抗炎药物 (NSAID)。
24. 如权利要求 20 所述的组合物, 其特征在于, 所述核心和肠溶包衣的至少一个还含有缓释剂。
25. 一种增强有此需要的个体中紧密连接部完整性的方法, 包括 : 向所述个体施用一组合物, 所述组合物含有有效量的甘油磷酸盐。
26. 如权利要求 25 所述的方法, 其特征在于, 所述组合物是肠溶包衣组合物, 包括:
 - (i) 核心, 所述核心包括治疗有效量的甘油磷酸盐 ; 和
 - (ii) 围绕核心的肠溶包衣。
27. 一种治疗或预防有此需要的个体中紧密连接部功能障碍相关疾病的方法, 包括 : 向所述个体施用一组合物, 所述组合物含有有效量的甘油磷酸盐。
28. 如权利要求 27 所述的方法, 其特征在于, 所述疾病选自下组 : 炎性疾病和癌症。
29. 如权利要求 28 所述的方法, 其特征在于, 所述炎性疾病选自下组 : 炎性肠病 (IBD)、克罗恩病、腹膜炎、口炎性腹泻、溃疡性结肠炎、多发性硬化、遗传性疾病, 视力减退、病毒感染, 细菌毒素、神经炎症, 肺组织完整性疾病, 淋巴细胞运输中的疾病以及其他的免疫疾病。
30. 如权利要求 28 所述的方法, 其特征在于, 所述甘油磷酸盐是甘油磷酸钙。
31. 如权利要求 28 所述的方法, 其特征在于, 所述组合物是肠溶包衣组合物, 包括:
 - (i) 核心, 所述核心包括治疗有效量的甘油磷酸盐 ; 和
 - (ii) 围绕核心的肠溶包衣。

治疗和防治肠道损伤、紧密连接部功能障碍相关疾病的方法和组合物

[0001] 相关申请的交叉引用

[0002] 本申请要求 2013 年 2 月 20 日提交的美国专利申请号为 NO. 61/766, 976 的优先权、2013 年 5 月 24 日提交的美国专利申请号为 NO. 61/827, 080 的优先权, 以及 2013 年 10 月 30 日提交的美国专利申请号为 NO. 61/897, 672 的优先权, 它们的内容通过引用的方式并入本申请。

技术领域

[0003] 本发明涉及治疗和预防肠道损伤的方法和组合物。具体地, 本发明提供利用甘油磷酸盐来减轻、缓解或预防个体肠道损伤 (比如, 锻炼引发的肠道损伤或摄入非类固醇抗炎药而引发或加重的肠道损伤) 的方法。本发明也涉及调控紧密连接部、预防或治疗紧密连接部功能障碍 (tight junction dysfunction) 相关疾病的方法和组合物。

背景技术

[0004] 人体以及动物体中的血流分配与氧气需求呈现某种功能关系, 而它氧气需求又依赖于活动。也就是说, 血流分配到不同的血管床, 供应氧需求最高的器官。这一目的通过限制血管供应低氧需求的器官以及松弛供应高氧需求器官的血管来实现。

[0005] 血流对血管壁有切应力的作用, 从而刺激合成细胞保护剂 (如一氧化氮和前列腺素)。中断或减少血流能够减少切应力, 从而减少这些物质的合成, 进而使得组织受到局部缺血的损害 (例如, 减少或限制血流引起的损害)。

[0006] 内脏循环 (splanchnic circulation) 是指血液循环到胃肠道器官, 包括胃、小肠、结肠、胰腺、肝脏和脾脏。内脏流 (血液流向肠道) 是高度可变的, 并且当流向肠道的血流量减少时, 肠道会在轻度到中度缺血损伤的条件下行使功能。

[0007] 尤其是, 剧烈运动期间的内脏流会被削减, 因为血流流向了骨骼肌、心脏和皮肤。因此, 在剧烈运动期间肠道常在轻度到中度缺血损伤的条件下行使功能。同时摄入布洛芬 (一种非类固醇抗炎药或 NSAID) 后, 这种损伤会进一步加剧 (详见 van Wijk 等, *Medicine&Science in Sports&Exercise*, 44, 2257-2262 (2012); 在下文称为“van Wijk”)。

[0008] NSAIDs 阻止介导疼痛和炎症的前列腺素的合成。然而, 前列腺素也是细胞保护剂, 对胃肠道的保护作用尤其明显。因此, 长期服用 NSAID 对胃肠 (GI) 道带来负面效果, 比如溃疡和肠道屏障功能受损, 结果增加了肠黏膜通透性 (E. Focalin, *Ann. Clin. Lab. Sci.* 2, 67-81 (1998))。

[0009] 运动员通常会寻找多种方式预防运动带来的身体疼痛, 随后改善他们的身体活动能力。运动员通常在体育活动前、期间和 / 或之后服用 NSAID 以治疗已有的肌肉骨骼痛, 或运动或体育活动预期带来或引发的肌肉骨骼痛。单独摄入 NSAID 就能够引起肠道损害。然而, 当与锻炼相结合时, NSAID 对肠道的损害, 尤其是对肠道屏障功能的损伤以及对肠黏膜通透性的增加会大大增强。

[0010] 肠道脂肪酸结合蛋白 (I-FABP) 是 15kDa 的蛋白, 该蛋白在血浆中出现是肠上皮细胞 (肠吸收性细胞) 损伤的标记。近年来, van Wijk 已经报道了当给正在进行中度运动的个体施用布洛芬时, 布洛芬能够加重运动诱发的小肠损伤, 尤其增加胃与十二指肠和小肠的通透性。该研究检测了在一给定的运动阶段 (周期) 之前、期间以及之后, I-FABP 在外周血浆中的出现情况。相比于仅运动的个体检测的血浆 I-FABP 水平, 当在运动前给个体施用 400mg 的布洛芬时, 血浆 I-FABP 水平显著升高。通过测定通常透不过肠道屏障的不可代谢单糖或二糖的吸收量, 评估得到个体的肠道通透性也显著增加的结论。总而言之, 运动和布洛芬均可以增加肠道的通透性。二者结合的效应高于单个效应的总和, 意味着二者通过不同的途径起作用, 二者可能均集中在肠上皮细胞活性以及肠上皮细胞 - 肠上皮细胞的屏障作用。

[0011] NSAID 通常被一般人群用于减轻微痛, 以及减少发热和炎症。另外, 尽管有风险, NSAID 通常被运动员用于治疗已有的肌肉骨骼痛, 以及预防任何潜在的运动伴随的或诱发的肌肉骨骼痛。对于一些特殊的运动, 参与者的服用剂量据报道高达 90% (T. Gorski 等, *Br. J. Sports Med.*, 45(2), 85-90 (2011); E. Taioli, *Br. J. Sports Med.*, 41(7), 439-441 (2007); W. V. Thuyne, *Clin. J. Sport Med.*, 18(2), 143-147 (2008))。

[0012] 上皮或上皮细胞之间的空间要密合, 使得从一层向另一层运输的分子不会经过这些空间扩散回来。紧密连接部在上皮和上皮细胞内形成了一屏障, 以控制水和溶质经细胞旁路空间运动。紧密连接部还通过形成“篱笆”来阻止顶端膜的分子和侧膜的分子混合在一起, 从而具有保持细胞极性的功能。在许多疾病中, 比如癌症或炎症疾病, 都出现了紧密连接部功能紊乱 (见 C. Forster; *Histochem Cell Biol.*; 130(1):55-70 (2008 年 7 月))。

[0013] 据报道, 鞘氨醇 1- 磷酸 (S1P) 是一种血清生物活性脂质, 激活紧密连接部相关的紧密连接蛋白 -1 (ZO-1), 进而在调控内皮细胞趋化性和屏障完整性方面起关键作用 (见 Lee 等, *J Biol Chem.*, 29; 281(39):29190-200 (2006 年 9 月); 电子公布 2006 年 8 月 6 日)。

[0014] 通常认为, NSAIDs 很可能通过两种机制来减少肠上皮细胞紧密连接部的“紧密性”, 从而导致增加了肠道的通透性。在美国, 一些作为非处方药出售的 NSAID 有抑制氧化磷酸化作用的副作用。因此, NSAID 干扰了细胞能量的产生。在这方面, 它们可能会加剧在运动期间观察到的血流减少流向肠道的效应。减少的血流会降低可用的氧, 而且 NSAID 会同时减弱细胞对可用氧的利用能力。

[0015] 近来的证据表明, 在培养的肠上皮细胞中, S1P 不仅控制特定紧密连接蛋白的表达, 还增加既有的紧密连接部的“紧密性” (Greenspon 等, *Dig. Dis. Sci.* 56, 1342-1353 (2011))。接触 S1P 后, S1P 对接合点完整性的影响在短短 30 分钟内就能观察到。S1P 由细胞鞘脂类 (鞘氨醇) 的磷酸化作用产生。它只有以磷酸化的形式 (即 S1P) 才能启动重要的调控, 即“信号传导”作用。然而, 磷酸化状态的 S1P 通过不断接触去磷酸化的酶, 而一直处于下调活性的状态。

[0016] 本领域迫切需要一种治疗和预防肠道损伤 (尤其是由摄入 NSAID 引起的或运动诱发的肠道损伤) 的组合物和方法。更具体地, 迫切需要一种治疗和预防运动诱发的肠道损伤的组合物和方法, 用于经常在运动前、运动时、或运动后立即服用 NSAID 以减缓和 / 或预防运动相关僵硬或疼痛的人们。更优的是, 该方法无毒、无害, 并且没有严重的副作用。本领域还需要调控紧密连接部的新方法和组合物, 以便预防或治疗与紧密连接部功能障碍相

关的疾病。

[0017] 发明概述

[0018] 本发明提供一种治疗或预防肠道损伤或其症状（包括摄入非固醇类抗炎药（NSAID）诱发或加重的肠道损伤、运动诱发的肠道损伤，以及为治疗或预防运动相关的肌肉骨骼疼痛而摄入的 NSAID 加重的运动诱发肠道损伤）的方法和组合物，根据本发明的实施方式，给个体施用甘油磷酸盐，尤其施用甘油磷酸钙，是减缓、缓解、和 / 或预防个体肠道损伤或其症状的有效方法。

[0019] 在一方面，本发明涉及一种治疗或预防个体肠道损伤或其症状的方法，所述方法包括向个体施用一组合物，所述组合物包括治疗有效量的甘油磷酸盐，从而治疗或预防肠道损伤或其症状。在一个实施方式中，肠道损伤或其症状由运动诱发，并且个体参与运动。较佳的，组合物经口腔或鼻腔给药。更佳地，组合物经口腔给药。

[0020] 在另一方面，本发明涉及一种治疗或预防个体中由非类固醇抗炎药物（NSAID）而加重的肠道损伤或其症状，其中，个体服用 NSAID，所述方法包括向个体施用一组合物，所述组合物包括治疗有效量的甘油磷酸盐，从而治疗或预防 NSAID 加重的肠道损伤或其症状。较佳的，组合物经口腔或鼻腔给药，更佳地，组合物经口腔给药。在一个实施方式中，肠道损伤或其症状由运动诱发，并且个体参与运动。

[0021] 根据本发明的实施方式，与个体未服用甘油磷酸盐导致的肠道损伤和 / 或其症状相比，向个体施用治疗有效量的甘油磷酸盐，尤其是甘油磷酸钙，导致肠道损伤和 / 或其一种或多种症状，比如那些运动诱发的症状的减缓、缓解或预防。与个体未服用甘油磷酸盐导致的肠道损伤和 / 或其症状相比，甘油磷酸盐，优选甘油磷酸钙，在减缓、缓解或预防由摄入 NSAID 进一步加重的肠道损伤和 / 或其一种或多种症状方面同样有效，比如，那些运动诱发的症状。

[0022] 本发明还涉及一种新的肠溶包衣组合物，所述组合物包括治疗有效量的甘油磷酸盐，以及治疗或预防个体肠道损伤，增加个体紧密连接部的完整性，以及治疗或预防个体紧密连接部功能障碍相关疾病的方法，包括向个体施用本发明所述的肠溶包衣组合物，比如递送位点特异性组合物。

[0023] 在一方面，本发明提供了一种肠溶包衣组合物，包括治疗有效量的甘油磷酸盐。根据本发明的实施方式，肠溶包衣组合物包括：核心，包括治疗有效量的甘油磷酸盐；以及包被核心的肠溶包衣。

[0024] 在一优选的实施方式中，肠溶包衣防止甘油磷酸盐从核心中释放出来，直到确认组合物至少进入个体小肠的空肠 / 回肠部分、以及大肠为止。

[0025] 在另一方面，本发明还涉及一种增强个体紧密连接部完整性的方法，所述方法包括向个体施用一组合物，所述组合物包括有效量的甘油磷酸盐。

[0026] 在另一方面，本发明还涉及一种治疗或预防个体紧密连接部功能障碍相关疾病的方法，所述方法包括向个体施用一组合物，所述组合物包括有效量的甘油磷酸盐。

[0027] 附图简述

[0028] 当与附图一起结合阅读时，本发明详述部分会更好理解。为了说明本发明，附图显示的是最佳实施方式。然而，应理解，本发明并不限于所示的实施方式。在附图中：

[0029] 图 1 显示了在有和没有浓度增加的 CGP 存在下，基线甘露醇通量与时间的关系

图；

[0030] 图 2 显示了在有和没有浓度增加的 CGP 存在下,CGP 对跨上皮电阻 (TEER) 的影响；

[0031] 图 3 显示了 CGP 对 Caco-2 细胞中鞘氨醇 1- 磷酸基线产量的影响；

[0032] 图 4 显示了向 Caco-2 细胞施用甘油磷酸钙 (CGP) 4 小时后,细胞内甘露醇渗透的增加量；

[0033] 图 5 显示了缺氧时跨上皮甘露醇通量图；

[0034] 图 6 显示了缺氧时的 TEER 图；

[0035] 图 7 显示了 CGP 对细胞因子诱导的跨上皮甘露醇通量的影响；和

[0036] 图 8 显示了细胞因子混合物 ((TNF- α)、(IFN- γ) 和 (IL- β)) 处理后,CGP 对跨上皮电阻的影响。

[0037] 发明详述

[0038] 除非有其他定义,否则本文所用的所有技术性和科学性术语是本领域技术人员普遍理解的相同含义。所有本文引用的出版物和专利通过引用的方式并入本文。包括在本发明说明书中的文件、法案、材料、设备、文章等等的讨论均以提供本发明的背景为目的。就所披露的或请求保护的任何发明而言,此类讨论并非承认任何或全部的这些材料构成现有技术的一部分。

[0039] 必须注意,如本文和附属的权利要求所用,单数形式的“一”、“某”、以及“该”包括复数引用,除非上下文另有明确的说明。

[0040] 如本文所用,术语“甘油磷酸盐”指任何盐,包括甘油磷酸根基团和无毒阳离子。无毒阳离子的非限制性例子包括钙、钠、钾和镁。适用于本发明的甘油磷酸盐包括(但不限于):甘油磷酸钙、甘油磷酸钠、甘油磷酸钾和甘油磷酸镁。较佳地,甘油磷酸盐为甘油磷酸钙。

[0041] 如本文所用,术语“甘油磷酸钙”或“CGP”指具有分子式 $C_3H_7CaO_6P$ 和分子量为 210.04 的无水形式的化合物。“CGP”也可以以水合物形式存在,包括一水合物和二水合物的形式。“CGP”也称为 1,2,3-丙三醇、单(二氢磷酸)钙盐(1:1)、甘油磷酸钙和 NEUROSIN®。CGP 存在三种同分异构体,分别为 β -甘油磷酸钙盐 ($HOCH_2)_2CHOP_3Ca$) 和 D(+)- α -甘油磷酸钙盐 ($HOCH_2CH(OH)CH_2OP_3Ca$)、L(-)- α -甘油磷酸钙盐 ($HOCH_2CH(OH)CH_2OP_3Ca$)。任何一种同分异构体,或任何两种或更多种同分异构体的组合可在本发明中用作 CGP。

[0042] CGP 可以用本领域已知的方法合成。CGP 还可以通过多种商业途径获得。商业上可获得的 CGP 的制备包括(但不限于):从 AkPharma Inc. 公司购得 (Pleasantville, NJ-08232),从 Astha Laboratories Pvt,Ltd 购得 (B-4, Industrial Estate, Sanathnagar, Hyderabad-18, 印度),Seppic Inc. 公司购得 (30Two Bridges Road, Fairfield, NJ 07004),以及从世界各地的许多其他的制造商和分销商中购得。

[0043] 如本文所述,术语“非类固醇抗炎药物”或“NSAID”指一类具有止痛(减轻疼痛)、退热(减轻发热)、和/或抗炎效果的化合物。NSAID 的例子包括(但不限于):布洛芬、阿司匹林、萘普生、非诺洛芬、酪洛芬、氟比洛芬、奥沙普秦、吲哚美辛、苏灵大、依托度酸、双氯芬酸、吡罗昔康、美洛昔康、替诺昔康、屈恶昔康、氯诺昔康、伊索昔康、以及其药学上可接受的盐。

[0044] 如本文所用,术语“运动”指任何形式的体育活动。在一实施方式中,运动可以指旨

在提高灵活性、或肌肉和关节的运动幅度的任何活动形式,比如拉伸。在另一实施方式中,运动可以指旨在增加心血管耐力的任何有氧运动形式,包括(但不限于):骑自行车、游泳、划船、跑步、徒步旅行和网球。在另一实施方式中,运动可以指旨在增加短期肌肉力量的任何有氧运动形式,比如,各个年龄段和比赛水平的举重训练、间歇训练、网球训练、橄榄球训练、足球、短跑、或任何其他的身体要求的运动或活动。尽管本发明方法可以治疗现披露的任何运动形式造成的肠道损伤,然而与无氧运动形式相比,肠道损伤更有可能在有氧运动后出现,因为在有氧运动中,氧的需求量更大,因而有氧运动后,对治疗的需求更大。

[0045] 如本文所用,术语“个体”指将要或已经施用本发明实施方式所述的组合物或化合物的任何动物,较佳地为哺乳动物,更佳地为人。哺乳动物的例子包括(但不限于):牛、马(更佳地,为赛马)、羊、猪、猫、狗、小鼠、大鼠、猫、狗、兔、豚鼠、猴、人,等等,更佳地,为人。

[0046] 如本文所用,术语“肠”指肠道,肠道包括小肠和大肠。小肠由十二指肠(即,胃近端最上面的部分),空肠(即,中部或中段),和回肠(即,大肠前的最后一段)组成。如本文所用,“十二指肠和空肠部分之间的连接部分”指十二指肠段和空肠段相接的小肠部分。如本文所用,“十二指肠和空肠部分之间的连接部分”指十二指肠段和空肠段相接的小肠部分。

[0047] 相对肠细胞通透性的肠屏障取决于毗邻肠细胞之间形成的紧密连接部。紧密连接部由蛋白链组成(主要是闭合蛋白和紧密连接蛋白),这些蛋白质围绕在肠腔附近的细胞周围。一个细胞的连接蛋白连接到邻近细胞的连接蛋白,在细胞之间形成致密结构,与榫槽接头将地板连接在一起的原理如出一辙。细胞之间紧密连接部的“紧密性”是许多蛋白链的直接功能体现,并且它们会受到细胞氧化状态以及细胞表面可溶性因子的影响。

[0048] 物质通过跨细胞路径(穿越细胞),或通过细胞旁路路径(细胞之间)穿过肠屏障。根据定义,在细胞之间穿梭的物质能够穿过紧密连接部。它们穿行的速度不仅取决于它们的分子量,还取决于紧密连接部的“紧密性”。

[0049] 如本文所用,术语“肠损伤”指肠受损,尤其是肠细胞受损。肠细胞是小肠和大肠粘膜中的主要细胞,负责营养物质、电解质和水的最终消化和吸收。“肠损伤”也指肠细胞之间连接部分受损,即,肠上皮细胞-肠上皮细胞的紧密连接部受损,由此导致正常的肠屏障功能被破坏、减弱或恶化。如本文所用,术语“肠损害”的范围应进一步包括肠通透性增加,该通透性增加是由于肠上皮细胞-肠上皮细胞的紧密连接部遭到破坏,进而破坏了肠上皮屏障的完整性,因此导致一些原本不能透过肠屏障的物质能够扩散穿越肠屏障。如本文所用,“肠损伤”还指肠损伤的症状、以及症状的发作,包括(但不限于):上腹部疼痛、肠胃气胀、消化不良以及嗝气。

[0050] 如本文所用,术语“肠道通透性增加”和“肠通透性增加”指肠细胞之间紧密连接部的“紧密性”降低。

[0051] 如本文所用,“治疗有效量”指能够有效减轻、缓解、降低肠损伤的程度,或预防肠损伤的甘油磷酸盐用量。“治疗有效量”同样也指能够有效减轻、缓解、降低肠损伤的程度,或预防任何肠损伤相关或表明肠损伤的症状的甘油磷酸盐用量。

[0052] 如本文所用,术语“治疗的”、“治疗”、或“疗法”指向个体施用一定量的甘油磷酸盐,其能够有效减轻、缓解、或预防个体肠道损伤和/或与肠损伤相关的一种或多种症状。

[0053] 如本文所用,术语“预防的”、“预防”、或“防治”指在肠损伤相关的症状发作之前,

向个体施用治疗有效量的甘油磷酸盐,从而能一起阻止任何的肠损伤或其相关症状,或推迟其出现,或仍可能,但是程度有所减轻(与不服用甘油磷酸盐的个体相比)。

[0054] 本发明的实施方式包括治疗或预防个体肠损伤的方法。由于在运动中,运动诱发血流从内脏循环(即,血液流向肠)改向以满足肌肉骨骼增加的氧需要量,从而导致肠通透性增加,因此运动本身也能够引起肠损伤。摄入 NSAID 可进一步加重或加剧运动引起的肠损伤。因此,本发明的实施方式还包括治疗或预防个体中运动诱发的肠损伤,治疗或预防由于个体摄入 NSAID 而加重的肠损伤,以及治疗或预防由于个体摄入 NSAID 而加重的运动诱发肠损伤。

[0055] 如本文所用,术语“运动诱发的肠损伤”指运动引起的肠损伤、或肠损伤的各种症状,从而运动诱发的肠损伤或肠损伤的各种症状比没有运动所致的肠损伤或肠损伤的各种症状要严重得多。如本文所用,术语“NSAID 加重的运动诱发的肠损伤”指摄入 NSAID 能够加重或加剧运动诱发的肠损伤或肠损伤的各种症状,从而运动诱发的肠损伤或肠损伤的各种症状比没有运动且摄入 NSAID 所诱发的肠损伤或肠损伤的各种症状要严重的多。

[0056] 治疗或预防肠损伤或各种症状的方法

[0057] 如本发明的实施方式所述,治疗或预防个体肠损伤或其症状的方法包括,向个体施用治疗有效量的甘油磷酸盐。在一实施方式中,治疗或预防的肠损伤或其症状是由运动诱发的。在另一实施方式中,治疗或预防的肠损伤或其症状是由运动诱发,并且由摄入 NSAID 进一步加重。

[0058] 因此,如本发明的实施方式所述,要给予有效量甘油磷酸盐的个体可以是进行体育运动的人,从而要治疗或预防的肠损伤或其症状是由运动诱发的。要给予有效量甘油磷酸盐的个体还可以是服用 NSAID 的人,从而要治疗或预防的肠损伤或其症状是由 NSAID 诱发的。在本发明中,要给予有效量甘油磷酸盐的个体可以进一步是既进行体育运动,又服用 NSAID 的人,比如,为了减轻目前的或预期的与运动相关的肌肉骨骼痛。可通过摄入 NSAID 治疗的运动相关肌肉骨骼痛的非限制性例子包括关节炎,如膝关节炎或肘关节炎;关节损伤,如扭伤;肌腱炎;韧带拉伤或撕裂,如前十字韧带撕裂(ACL);胫骨应力综合症(外胫夹);足底筋膜炎;以及扭伤腿筋、四头肌、臀肌、肱二头肌、或肱三头肌(抽筋)。能够通过摄入 NSAID 治疗的运动相关肌肉骨骼痛还包括运动后的单纯肌肉和关节疼痛,没有特别的创伤经历或表征。就本发明的目的而言,不直接关注 NSAID 能够缓解体内任何部位的感知的或预期的疼痛的实际有效性,本发明关注的是运动者在运动或接近运动的时段服用 NSAID 会将肠细胞置于特定的危险中这一事实。

[0059] 如本发明所述,摄入 NSAID 和从事体育锻炼不需要同时进行,然而,只有同时进行时,有效量的 NSAID 才会存在于马上要运动的或在运动初始阶段的个体体内,因而有可能在运动开始时加剧或加重运动自身诱发的任何肠损伤。例如,NSAID,如布洛芬可在运动(如跑步)前 60 分钟被服用。

[0060] 在本发明的一个实施方式中,施用治疗有效量的甘油磷酸盐能够带来减轻、缓解、或预防一种或多种肠损伤症状方面的临床上可见的有益效果,从而使得,与个体没有接受治疗有效量的甘油磷酸盐时所述肠损伤所导致的症状相比,施用后的肠损伤症状是约 90%、80%、70%、60%、50%、40%、30%、20%、10%或更少。

[0061] 在本发明的另一实施方式中,施用治疗有效量的甘油磷酸盐能够带来在减轻、缓

解、或预防一种或多种由 NSAID 加重的肠损伤症状方面的临床上可见的有益效果,从而使得,与个体没有接受治疗有效量的甘油磷酸盐时,摄入 NSAID 导致的症状相比,施用后的肠损伤症状是约 90%、80%、70%、60%、50%、40%、30%、20%、10%或更少。

[0062] 在本发明的另一实施方式中,施用治疗有效量的甘油磷酸盐能够带来在减轻、缓解、或预防一种或多种运动诱发的肠损伤症状方面的临床上可见的有益效果,从而使得,与个体没有接受治疗有效量的甘油磷酸盐时,仅仅由运动导致的症状相比,施用后的肠损伤症状是约 90%、80%、70%、60%、50%、40%、30%、20%、10%或更少。

[0063] 在本发明的另一实施方式中,施用治疗有效量的甘油磷酸盐能够带来在减轻、缓解、或预防一种或多种由 NSAID 加重的,运动诱发的肠损伤症状方面的临床上可见的有益效果,从而使得,与个体没有接受治疗有效量的甘油磷酸盐时,运动并摄入 NSAID 导致的症状相比,施用后的肠损伤症状是约 90%、80%、70%、60%、50%、40%、30%、20%、10%或更少。

[0064] 在本发明的另一实施方式中,临床上可见的有益效果可以是以下情况:与个体没有接受治疗有效量的甘油磷酸盐时所导致的症状相比,阻止了肠损伤、由 NSAID 加重的肠损伤、运动诱发的肠损伤或由 NSAID 加重的运动诱发的肠损伤、和 / 或其一种或多种症状的进一步恶化或发展,或随后其程度减轻。

[0065] 在本发明的另一实施方式中,临床上可见的有益效果可以是以下情况:阻止肠损伤、由 NSAID 加重的肠损伤、运动诱发的肠损伤或由 NSAID 加重的运动诱发的肠损伤、和 / 或其一种或多种症状的出现,从而使个体不经历在个体没有接受治疗有效量的甘油磷酸盐时所导致的任何肠损伤或其症状。

[0066] 在一优选的实施方式中,施用于个体的甘油磷酸盐是甘油磷酸钙。

[0067] 鉴于本发明公开的内容,用标准程序评估服用甘油磷酸盐对个体的效果,从而让技术人员确定个体服用的甘油磷酸盐的有效量。甘油磷酸盐在临床上的有益效果对于个体是能够明显观察到的。例如,与服用 NSAID、且未服用甘油磷酸盐的个体所经历的消化不良、上腹部疼痛或肠胃气胀的程度相比,在运动前也服用 NSAID 的服用有效量甘油磷酸盐的个体随后在运动期间或运动后不会出现消化不良、上腹部疼痛、或肠胃气胀。可在个体中直接观察到的症状还包括便秘或腹泻或炎症症状,如关节疼痛、肌肉压痛等。评估的症状还可类似于剧烈运动后通常可以估计到的肌肉骨骼综合症,如肌肉或关节疼痛等。

[0068] 还可以通过临床检验来确定甘油磷酸盐的临床上的有益效果,以评估甘油磷酸盐在维护肠屏障完整性中的效果,从而治疗或预防肠损伤。在这种情况下,可以客观评定临床上的有益效果,并且临床上的有益效果可以不需要从个体上直接观察到或能够从个体上很明显看到。例如,肠上皮细胞受损(比如可能由于可用的氧和 / 或氧的利用被破坏所致)从细胞中释放出肠脂肪酸结合蛋白(I-FABP)。I-FABP 继而进入循环系统,其在血浆中能够被检测到。因此,血浆 I-FABP 是肠细胞损伤的标记物,并且 I-FABP 的水平能够很容易被检测到,比如,通过酶联免疫吸附分析(ELISA)来确定甘油磷酸盐对减轻肠损伤的效果。另一个评价肠损伤和甘油磷酸盐在预防或减轻肠损伤效果的临床实验的例子是多糖分析,如 J. Chromatog. B Analyt. Tech. Biomed. Life Sci., 879(26), 2794-2801(2011) 中所描述的,该文献通过引用的方式纳入本文。多糖分析通过测定非代谢单糖或二糖的摄入和尿排泄来测定肠细胞的通透性。这些糖通过细胞旁路路径(细胞之间)被吸收,然而此种情况仅仅

当肠上皮细胞 - 肠上皮细胞连接部的紧密性被降低时才能够发生。

[0069] 施用于个体以提供治疗有效量的甘油磷酸盐的甘油磷酸盐剂量不限于具体的数值。本领域的普通技术人员会认识到,观察到临床上有益效果所需的甘油磷酸盐的治疗有效量还依赖于额外的因素,比如需要治疗的个体的体重、年龄、性别等,以及他们肠道的初始健康状态中,本领域技术人员不难确定所需提供的治疗有效量的合适剂量。在确定根据本发明施用于个体的甘油磷酸盐给药方案和有效治疗量时需要考虑的额外因素包括(但不限于):个体对 NSAID 的敏感性以及个体参与运动的强度和规律性。例如,为了治疗或预防个体中摄入 NSAID 而加重的,运动诱发的肠损伤,可给予个体 CGP。相对于 NSAID,CGP 的剂量可涵盖很广的范围,较佳地,CGP 与 NSAID 以摩尔比为 0.1:1-10:1 给予。作为一个说明性的例子,如果使用的 NSAID 为布洛芬,考虑到 CGP 与 NSAID 有近乎相同的分子量($MW_{CGP} = 210.04$; $MW_{布洛芬} = 206.29g/mol$),那么典型的剂量比为 CGP 与布洛芬的重量比为 1:1。在这种情况下,对于 200mg 剂量的布洛芬可以给予 200mg 的 CGP。同样地,对于 400mg 剂量的布洛芬可以给予 400mg 的 CGP,等等。如果阿司匹林($MW = 189g/mol$)作为 NSAID 施用,当阿司匹林的施用剂量为 650mg 时,相应的 CGP 的剂量可以是 800mg。对于其他的 NSAID,CGP 的剂量同样地与推荐的或典型的 NSAID 剂量相匹配。在不存在 NSAID 的情况下,向个体施用 CGP 或其他甘油磷酸盐,以治疗或预防运动诱发的肠损伤,CGP 或其他甘油磷酸盐的剂量可以与个体摄入 NSAID 时的剂量相同。

[0070] 甘油磷酸盐和 NSAID 可以在单一的组合物中给予,比如组合片剂或胶囊,或它们可以在不同的组合物中给予,比如两个单独的药片或胶囊。在任何一种情况下,甘油磷酸盐和 NSAID 均不需要一起摄入,然而必须同时摄入,从而使得甘油磷酸盐和 NSAID 都能够彼此同时接触脆弱的肠细胞。

[0071] 根据本发明的一个实施方式,治疗有效量的甘油磷酸盐以固体或液体的形式,例如粉末或液体混合物的形式口服施用于个体。当以固体的形式口服给药时,甘油磷酸盐可以,例如,以粉末、药片、胶囊、囊片、囊形片、或液体胶囊的形式给药。当以液体的形式口服给药时,甘油磷酸盐可以,例如,以乳剂、溶液、悬浮液、糖浆、酏剂的形式给药。

[0072] 根据本发明的另一个实施方式,治疗有效量的甘油磷酸盐作为固体粉末或液体混合物以喷鼻剂/吸入混合物经鼻腔施用于个体。当作为喷鼻剂/吸入混合物经鼻腔给药时,甘油磷酸盐可以是颗粒、或作为溶解/悬浮在水载体溶液/悬浮液中的液体乳液、溶液糖浆、或酏剂的形式。在本实施方式中,不需要缓释。

[0073] 在一优选的实施方式中,甘油磷酸盐可以口服。虽然口服是较佳方式,因为鼻腔给药不是到达肠道的直接途径,但由于鼻膜的实质性吸收水平,一些鼻腔给药的产品很容易经由向下通过食道的后区上呼吸道引流(rear area upper respiratory drainage)而被吞咽,藉此直接进入肠道,尽管经由该途径对于通过胃肠道行进的部分而言没有缓释作用。

[0074] 根据本发明的另一实施方式,甘油磷酸盐可以结合体育定向(sports-directed)食品或饮料口服施用于个体。如本文所用,术语“体育定向食品或饮料”指任何在市场上明确标示卖给运动员的饮料或食品,其能够提高运动员的表现能力,或从运动、训练、或任何与运动或田径表演相关的活动中恢复。体育定向食品或饮料的非限制性例子包括能量条、蛋白粉、高蛋白饮品、和运动饮料,尤其是那些出于运动后辅助水化功能或替换电解质而设计的食品或饮料。甘油磷酸盐可以溶解、或悬浮在体育指导型饮料中,在个体摄入体育指导

型饮料后,以口服的形式将甘油磷酸盐施用于个体。甘油磷酸盐还可以与体育定向食品联用,从而在个体摄入体育定向食品后,以口服的形式将甘油磷酸盐施用于个体。例如,CGP 粉末可以与蛋白粉混合,或分解或悬浮在能量棒 (protein bar) 中。

[0075] 在本发明中,可采用本领域技术人员已知的方法配制含有治疗有效量的甘油磷酸盐的组合物以便用于本发明。为了制备施用于个体的组合物,根据传统的药剂配方技术将甘油磷酸盐与药学上可接受的载体混合。以组合物的总重计,用于本发明的组合物可以包括治疗有效量的甘油磷酸盐,其含有约 10-90% 的甘油磷酸盐,较佳地,50-90%,更佳地,75-90%。尽管组合物的活性部分较佳地含有 100% 的甘油磷酸盐,然而甘油磷酸盐在全部配制化合物中的比例与相关的保护性包衣的重量呈函数关系,以确保活性盐能够到达小肠和大肠的合适部位。甘油磷酸盐的重量取决于甘油磷酸盐的具体阳离子。较佳地,其核心占组合物的至少 95%,余量为保护性包衣。然而,这些百分比不是限制性的,并且可以用常规实验予以确定。

[0076] 药学上可接受的载体可以包括一种或多种赋形剂,如粘结剂、悬浮剂、乳化剂、润湿剂、润滑剂、调味剂、甜味剂、防腐剂、染料、以及涂层。载体可以采取各种形式,取决于给药的制剂形式。对于液体口服制剂,例如,悬浮剂、酏剂和溶液剂,合适的载体和添加剂包括水、乙二醇、油、酒精、调味剂、防腐剂、着色剂等。对于固体口服制剂,例如,粉末、胶囊、囊片、软胶囊和片剂,合适的载体和添加剂包括淀粉、糖、稀释剂、颗粒剂、润滑剂、粘结剂、崩解剂等。对于喷鼻剂/吸入混合物,水溶液/悬浮液可以包括水、乙二醇、油、软化剂、稳定剂、润湿剂、防腐剂、芳香剂、调味剂等作为合适的载体和添加剂。

[0077] 水中 CGP 的溶解度大约为 1g/100mL。因此,为口服给药,可以制备 CGP 的溶液/悬浮液,如本发明所述,例如,在水中溶解/分解和悬浮 CGP 的粉末或片剂。如果悬浮液可以接受,则可以使用大于 1% 的 CPG,并且使用任意量的常用、已知的稳定剂使之保持悬浮状态,所述稳定剂包括(但不限于):琼脂、羧甲基纤维素钠、瓜儿胶等。在一具体的实施方式中,CGP 粉末或片剂可以溶解在体育定向饮料中,或崩解、悬浮或混合在体育定向食物中。在本发明中,CGP 还可以制备成缓释的形式,以适合使用几个小时。本领域技术人员知晓制备缓释剂的方法。喷鼻剂的典型施用方法如下:根据个体摄入的 NSAID 量,按需要,每天每个鼻孔喷雾 1-2 次,每天不止一次。包含其他甘油磷酸盐,例如甘油磷酸钠、甘油磷酸镁或甘油磷酸钾的组合物可以类似于 CGP 组合物制备。

[0078] 根据本发明的实施方式,可以在任何时间点向个体施用甘油磷酸盐,以治疗或预防肠损伤。例如,甘油磷酸盐可以一天施用 1 次或 2 次,以治疗或预防肠损伤,或根据需要,可以更频繁的施用,没有不良影响。

[0079] 根据本发明的实施方式,可以在任何时间点向个体施用甘油磷酸盐,以治疗或预防由 NSAID 加重的肠损伤,前提是,甘油磷酸盐的治疗效果与 NSAID 的给药及其在加重肠损伤方面的作用重叠,由此可以观察到在临床上的有益效果。甘油磷酸盐可以,例如与 NSAID 同时给药,在摄入 NSAID 之前立即给药,或在摄入 NSAID 后立即给药。

[0080] 根据本发明的实施方式,可以在任何时间点向个体施用甘油磷酸盐,以治疗或预防运动诱发的肠损伤,前提是,甘油磷酸盐的治疗效果与运动诱导的肠损伤发作或持续期重叠,由此可以观察到临床上的有益效果。较佳地,甘油磷酸盐在运动开始前、运动期间、或运动后立即给药。

[0081] 根据本发明的实施方式,可以在任何时间点向个体施用甘油磷酸盐,以治疗或预防由 NSAID 加重的运动诱发的肠损伤,前提是,甘油磷酸盐的治疗效果与 NSAID 的给药及其在加重肠损伤方面的作用重叠,由此可以观察到临床上的有益效果。甘油磷酸盐可以在,例如摄入 NSAID 后但运动开始前给药,或甘油磷酸盐可以在摄入 NSAID 后、运动期间给药。甘油磷酸盐可以在摄入 NSAID 和运动之前给药。甘油磷酸盐还可以在摄入 NSAID 之后、运动结束后给药,或在运动初始前,与 NSAID 一起给药。

[0082] 由于甘油磷酸盐,尤其是 CGP 无毒、无害,没有已知的副作用,甘油磷酸盐给药的次数可以不受限制。因此,甘油磷酸盐还可以一天给药数次,比如,一天给药一次、两次或三次、或更多次。例如,当治疗或预防运动诱发的肠损伤时,甘油磷酸盐(如 CGP)可以给药数次,比方说在运动前或运动后,或运动前、运动期间、和运动后予以给药。如果是为了预防由 NSAID 加重的肠损伤,甘油磷酸盐可以在每次 NSAID 摄入后给药。如果是为了预防任何预期的由 NSAID 造成加重的运动诱发的肠损伤及其相关症状,甘油磷酸盐宜在摄入 NSAID 之前、或与 NSAID 同时给药。上述情况应是非限制性例子,并且应理解,本领域的普通技术人员会认识到,当施用 CGP 或其他甘油磷酸盐时,尤其是相对于摄入 NSAID 或 CGP 或其他甘油磷酸盐,更尤其是相对于摄入 NSAID 和参与运动施用 CGP 或其他甘油磷酸盐时,会产生本发明所述的临床上的有益效果。

[0083] 本发明所使用的,含有治疗有效量的甘油磷酸盐的组合物,可以任选的包括额外的治疗性添加剂,所述治疗性添加剂包括 NSAID,具体如上所述。因此,在本发明的一优选实施方式中,甘油磷酸盐和 NSAID 可以作为同一组分的一部分,同时施用于个体。较佳地,甘油磷酸盐是 CGP。本发明所使用的 CGP 和 NSAID 组合组合物优选含有一定量的 CGP,其重量是 CGP 和 NSAID 的混合物中 NSAID 重量的大概 10% -1000%,较佳地,30-70%,更佳地,40-60%,最佳地,为组合物中 NSAID 重量的 50%。当与 NSAID 同时施用,并且不是含 NSAID 的共同组合物的一部分时,CGP 与 NSAID 的重量比宜为 1:1。在一个优选的实施方式中,NSAID 为布洛芬。作为示范性而非限制性例子,含有 CGP 和布洛芬的组合物可以在运动(如跑步)前 60 分钟施用于个体,以治疗和/或预防本发明所述的运动诱发的肠损伤。

[0084] 为了减轻运动相关的肌肉骨骼痛,而长期在运动前、运动期间、或运动后立即摄入 NSAID 的个体可以是经历严格训练的运动员,其几乎每天都需要强化训练。对于这类个体,需要甘油磷酸盐的缓释剂型来治疗或预防由 NSAID 加重的运动诱发的肠损伤。使用甘油磷酸盐的缓释剂型可以限制甘油磷酸盐的施用次数,还不影响治疗效果。然而,如上所述,甘油磷酸盐,尤其是 CGP 是无毒、无害,且没有已知的副作用的,因此,开出甘油磷酸盐缓释剂型处方的个体可以任选增补标准甘油磷酸盐配方的给药方案,以便最大程度提高治疗效果。较佳地,本发明所使用的延长释放或缓释制剂含有 CGP。

[0085] 增强紧密连接部完整性和治疗或预防紧密连接部功能障碍相关疾病的方法

[0086] 本发明意外地发现,甘油磷酸盐,如甘油磷酸钙(CGP),增强了紧密连接部的完整性,并且降低了跨膜通透性。

[0087] 相应的,本发明的一方面涉及一种增强有此需要的个体中紧密连接部完整性的方法,所述方法包括:向个体施用一组合物,所述组合物含有有效量的甘油磷酸盐,如甘油磷酸钙(CGP)。甘油磷酸盐有效量的确定、施用效果、施用方法、以及组合物中的组分已经在之前的治疗肠损伤或其症状的方法部分有过相应描述。

[0088] 本发明的另一方面涉及一种治疗或预防有此需要的个体中紧密连接部功能障碍相关疾病的方法。所述方法包括：向个体施用一组合物，所述组合物含有有效量的甘油磷酸盐，如甘油磷酸钙（CGP）。甘油磷酸盐有效量的确定、施用效果、施用方法、以及组合物中的组分已经在之前的治疗肠损伤或其症状的方法部分有过相应描述。

[0089] 任何与紧密连接部功能障碍相关的疾病可以用本发明的方法予以预防或治疗。

[0090] 在本发明的一个实施方式中，与紧密连接部功能障碍相关的疾病是癌症、如乳腺癌（例如，浸润性导管癌）；前列腺癌（例如，前列腺腺癌）；甲状腺癌、滤泡性腺瘤；胃食管反流疾病（例如，巴雷特食管症（不典型增生））；肺癌（例如，基底样鳞状细胞癌）等。（见 C. Foster, *Histochem Cell Biol.* ;130(1):55-70(2008 年 7 月)，其内容通过引用的方式全文并入本文）。

[0091] 在本发明的另一实施方式中，与紧密连接部功能障碍相关的疾病是炎症疾病，包括（但不限于）：炎性肠病（IBD），如胶原性结肠炎克罗恩病；炎性腹泻和克罗恩病、溃疡性结肠炎、多发性硬化；遗传性疾病，如遗传性耳聋、家族性低镁症、神经炎症（见 Grin' Kina 等, *PLoS ONE* 7(5):e36475. doi:10.1371/journal.pone.0036475(2012)，其内容通过引用的方式全文并入本文），囊性纤维化；视力丧失，如糖尿病眼部疾病：糖尿病视网膜病变；病毒感染，如反转录病毒感染（脑积水、脑炎）；细菌毒素，如产气荚膜梭菌肠毒素；肺组织完整性疾病；淋巴细胞运输中的疾病（见 Mandala 等, *Science* 296, 346(2002)，其内容通过引用的方式全文并入本文），或其他免疫疾病（见 Rosen 等, *Nature Reviews Immunology* 5, 560-570(2005 年 7 月)，其内容通过引用的方式全文并入本文），等。在一优选的实施方式中，本发明涉及一种预防或治疗有此需要的个体中炎性肠病（IBD）的方法，所述方法包括：向个体施用一组合物，所述组合物含有有效量的甘油磷酸盐，如甘油磷酸钙（CGP）。更佳地，IBD 是胶原性结肠炎克罗恩病或克罗恩病，其主要症状为腹泻，主因为上皮屏障功能性障碍，由此导致溶质损失不断增加的“漏通量腹泻”。

[0092] 尽管不希望束缚于理论，然而通常认为，甘油磷酸盐，如甘油磷酸钙（CGP）提高了鞘氨醇-1-磷酸（S1P）的浓度和/或其在上皮或内皮细胞中的活性，由此降低跨上皮通透性。不希望束缚于理论，还据信，甘油磷酸盐至少部分通过抑制磷酸酶的作用而发挥作用，包括使得 S1P 去磷酸化的那些酶，从而导致甘油磷酸盐介导的 S1P 形成和长久性，并且增加 NSAID 和运动诱发的局部缺血攻击的肠道中的可用 S1P，从而维持连接部完整性。

[0093] 肠溶包衣组合物

[0094] 本发明还提供了一种肠溶包衣组合物，所述组合物包括治疗有效量的甘油磷酸盐。根据本发明的实施方式，本发明所述的肠溶包衣组合物是缓释制剂，可以用于治疗肠损伤、由 NSAID 加重的肠损伤、运动诱发的肠损伤、以及 NSAID 进一步加重的运动诱发的肠损伤。因此，本发明的实施方式还包括治疗或预防个体中肠损伤或其症状的方法，所述方法包括向个体施用本发明所述的肠溶包衣组合物。本发明的实施方式还包括增强个体中紧密连接部完整性的方法，以及治疗或预防个体中与紧密连接部功能障碍相关疾病的方法，所述方法包括向个体施用本发明所述的肠溶包衣组合物。

[0095] 根据本发明的实施方式，肠溶包衣组合物包括：(i) 核心，所述核心包括治疗有效量的甘油磷酸盐；和 (ii) 围绕在核心周围的肠溶包衣。在一个优选的实施方式中，甘油磷酸盐为甘油磷酸钙。

[0096] 如本文所用,术语“肠溶包衣组合物”指这样一种组合物,在该组合物核心的外面,围绕或包围着一层肠溶包衣,其中,所述核心包括治疗或药理活性化合物。如本文所用,术语“核心”指肠溶包衣组合物的组分,其含有治疗活性成分。本发明所述的肠溶包衣组合物中的核心最好制备成口服给药的固体剂型,比如,粉剂、片剂、胶囊、囊片、囊形片、液体凝胶、或颗粒剂等。由于本发明所述的组合物的核心为肠溶包衣所包围或包被,该核心为组合物的内层或组合物的一部分。

[0097] 如本文所用,术语“肠溶包衣”指围绕或包被在组合物核心周围的一层,并且是组合物的最外层结构。较佳地,肠溶包衣完全包围或包被核心,由此使得向个体给药后,核心不会暴露于体液(如唾液、胃肠道液)中,直到肠溶包衣溶解或崩解为止。肠溶包衣防止组合物核心中的治疗或药学上的活性成分在肠溶衣分解、溶解或崩解之前释放出来,而是使得治疗活性成分在肠溶包衣溶解或分解、或开始溶解或分解时从核心中释放出来。较佳地,肠溶包衣没有分解、溶解或崩解,因此,来自核心的治疗活性成分直到组合物进入肠内才释放出来,更佳地,来自核心的治疗活性成分直到组合物进入个体小肠的空肠或回肠,以及实质性到达大肠时才会定量的释放出来。在一具体的实施方式中,肠溶包衣使得一旦组合物到达小肠的十二指肠和空肠的交界处,即释放来自核心的治疗活性成分,由此使得治疗活性成分在空肠部分开始释放,并且直到组合物到达远端小肠(回肠)以及大肠中才有效的释放。

[0098] 本发明所述的肠溶包衣组合物的肠溶包衣可以用于控制组合物核心中甘油磷酸盐在胃肠道中释放的位置,由此甘油磷酸盐是位点特异性的递送。如本文所用,术语“位点特异性的递送”指组合物核心中的治疗活性成分在所需的递送位点释放。根据本发明的实施方式,肠溶包衣组合物位点特异性地将甘油磷酸盐递送到肠中,较佳地,递送到小肠或大肠,更佳地,递送到小肠,最佳地,递送到小肠的十二指肠和空肠部分的交界处。因此,肠溶包衣使得组合物通过个体的胃转移,却不释放甘油磷酸盐,并且只有在组合物进入肠道时,肠溶包衣才开始分解或溶解,甘油磷酸盐才会被释放出来。

[0099] 在一优选的实施方式中,一旦本发明所述的肠溶包衣组合物的肠溶包衣分解或溶解,实质上所有的甘油磷酸盐几乎同时从组合物的核心中释放出来。在一更为优选的实施方式中,在组合物到达个体的小肠时,实质上所有的甘油磷酸盐会从组合物的核心中释放出来。

[0100] 在一个实施方式中,本发明所述的肠溶包衣组合物能够位点特异性地将甘油磷酸盐递送给小肠。当位点特异性地递送给小肠时,所述递送可以是递送到小肠的十二指肠、空肠、或回肠部分。在另一实施方式中,可以位点特异性地递送给大肠。较佳地,本发明所述的肠溶包衣组合物能够位点特异性地将甘油磷酸盐递送给十二指肠和空肠的交界处,由此使得,直到组合物到达至少空肠,才会开始甘油磷酸盐的最大释放,最大的可能是,它不会像营养物质一样吸收入体内,而是进入小肠的回肠和大肠后继续前行,对这些部位进行冲洗。

[0101] 根据本发明的实施方式,肠溶包衣由聚合材料组成。较佳地,聚合材料对 pH 敏感,由此使得聚合材料在高酸的 pH 条件下依然保持完整或结构稳定,但在弱酸 pH 条件下则会崩解、溶解或分解。本发明所使用的对 pH 敏感的合适聚合物为在胃的低 pH 环境(即, pH 大概为 3)中依然能具有完整性和稳定性,而在肠道的常见 pH 环境下(即,大概 pH 为 5-7)会分解或溶解。

[0102] 根据本发明的实施方式, 聚合材料可以是合成材料, 如丙烯酸或马来酸基聚合物和聚乙烯衍生物, 或有机材料, 如纤维素基聚合物。较佳地, 聚合材料对 pH 敏感。本发明所用的对 pH 敏感的丙烯酸聚合物的例子包括 (但不限于): 甲基丙烯酸的聚合物 (均聚物或共聚物)、甲基丙烯酸甲酯和丙烯酸乙酯等, 如, (甲基丙烯酸 / 丙烯酸乙酯) 共聚物, (甲基丙烯酸 / 甲基丙烯酸甲酯) 共聚物。甲基丙烯酸和丙烯酸乙酯的共聚物可以通过商业途径获得, 其商标名为 Eudragit® L-30-D 55, 可获自 Evonik Industries。本发明所用的对 pH 敏感的纤维素聚合物的例子包括 (但不限于): 羧丙基甲基纤维素邻苯二甲酸酯 (HPMCP)、羟丙基甲基纤维素醋酸琥珀酸 (HPMCAS)、羧甲基乙基纤维素 (CMEC)、邻苯二甲酸乙酸纤维素 (CAP)、醋酸琥珀酸纤维素 (CAS) 和醋酸纤维素偏苯三酸 (CAT)。本发明所用的其他商业上可获得的聚合物和肠溶包衣系统包括 Acryl-EZE®、Sureteric®、Nutraferic® II 和 Opadry® 肠溶包衣, 均获自 Colorcon 公司。

[0103] 根据本发明的实施方式, 基于本发明, 肠溶包衣可以包括任何聚合物材料、或其组合。聚合物材料可以占 (相对于肠溶包衣总重) 大概 5-90 重量%, 比如, 10%、20%、30%、40%、50%、60%、70%、80%、或 90%。

[0104] 根据本发明的实施方式, 肠溶包衣能够稳定存在于胃的酸性环境中 (该环境的 pH 约为 3), 却在 pH 约为 5 或更高的弱酸环境中溶解或分解, (比如肠道中的 pH 环境) 溶解或分解。例如, 肠溶包衣可以在 pH 为约 1、1.5、2、2.5、3、3.5 或 4、4.5、或 5 下稳定存在, 却在 pH 约为 5.5、6、6.5、7、7.5、8、8.5 或 9 下溶解或分解。

[0105] 本发明所述的肠溶包衣还可以进一步包括添加剂, 以改善肠溶包衣的性质, 比如塑化剂、润滑剂、染料、着色剂、消泡剂、乳化剂、表面活性剂等。例如, 塑化剂可以增加肠溶包衣的挠性和弹性, 降低肠溶包衣的脆性。塑化剂可以是亲水性增塑剂, 可以是疏水性增塑剂。用在本发明肠溶包衣上的增塑剂的例子包括 (但不限于): 邻苯二甲酸衍生物、邻苯二甲酸正丁酯、丙二醇、三醋精、硅油、邻苯二甲酸二乙酯、柠檬酸三乙酯、癸二酸二丁酯、和聚乙二醇等。用于肠溶包衣的润滑剂的例子包括硬脂酸镁、云母等。

[0106] 在一个优选的实施方式中, 肠溶包衣进一步包括塑化剂。塑化剂可以占相对肠溶包衣总重量的 5-60 重量%, 比如, 大概 10%、20%、30%、40%、50% 或 60%。

[0107] 肠道内的 pH 逐步升高, 从小肠上端 (如十二指肠) 的 pH 约为 5 升至小肠末端 (如回肠) 和大肠的 pH 约为 7.0-8.0。本发明所述的肠溶包衣组合物应配制: 肠溶包衣在组合物通过胃、以及至少到达小肠上端后才会溶解并释放甘油磷酸盐。因此, 肠溶包衣可以在十二指肠的 pH 范围内开始溶解, 并且在小肠的 pH 范围内继续溶解。肠溶包衣还可以在组合物进入小肠的末端 (即回肠) 或大肠时才开始溶解。

[0108] 较佳地, 在组合物至少进入个体的小肠或大肠时, 本发明所述的肠溶包衣组合物才允许甘油磷酸盐从核心中定量释放出来。更佳地, 在组合物至少进入小肠的空肠部分时, 本发明所述的肠溶包衣组合物才允许甘油磷酸盐从核心中定量释放出来。

[0109] 甘油磷酸盐从本发明所述的肠溶包衣组合物中释放出来的位置, 可以通过改变参数的方法予以调整, 所述参数包括肠溶包衣的厚度、肠溶包衣的聚合物的种类、肠溶包衣的 pH 敏感性、添加的添加剂等。较佳地, 肠溶包衣基本上在穿越肠道期间溶解, 这使得甘油磷酸盐在肠道中 (较佳地, 在空肠或回肠) 能够最大化的释放。

[0110] 在一个实施方式中, 肠溶包衣在 pH 约为 5.5 下溶解, 并且会位点特异性地将甘油

磷酸盐输送给小肠的十二指肠部分。在另一实施方式中,肠溶包衣在 pH 约为 5.5-7.0 下溶解,并且会位点特异性地将甘油磷酸盐输送给肠的空肠或回肠部分。在另一实施方式中,肠溶包衣在 pH 约为 7.0 下溶解,并且会位点特异性地将甘油磷酸盐输送给大肠。较佳地,肠溶包衣在 pH 约为 5.5-7.0 下溶解。

[0111] 根据本发明的实施方式,相对于核心,肠溶包衣的重量比约为 5-60%,较佳地,5-30%,比如,10%、25%、20%或 25% (例如,相对于核心的总重,肠溶包衣为 5 重量%)。然而,本领域的普通技术人员应认识到,肠溶包衣的重量比,以及组合物本身的重量比可以根据甘油磷酸盐释放的位置予以调整。例如,相对核心的约为 5-30% 的重量比适合于进入小肠的十二指肠和空肠部分,但是,进入小肠回肠或大肠的重量比可以高于 30%。

[0112] 根据本发明的实施方式,肠溶包衣的厚度和 / 或工艺参数 (如层数、聚合物的类型等) 能够影响肠溶包衣的溶解速度,并且因此影响甘油磷酸盐从核心释放的位置 (即,位点特异性释放)。如本文所用,术语“薄”和“厚”不仅包括涂层的厚度,还包括工艺参数 (sophistication)。例如,薄的肠溶包衣会比厚的肠溶包衣溶解或分解得更快,并且从含有薄的肠溶包衣的组合物中释放出来的甘油磷酸盐能够在更接近胃的肠道的位置发挥作用,比如十二指肠,而从含有厚的肠溶包衣的组合物中释放出来的甘油磷酸盐在胃的更末端的位置发挥作用,如空肠、回肠或大肠。

[0113] 通过调整肠溶包衣的层数,从而控制本发明所述肠溶组合物的肠溶包衣的厚度。在一个实施方式中,肠溶包衣组合物包括一层肠溶包衣。在另一实施方式中,肠溶包衣组合物包括一层以上的肠溶包衣,比如,1、2、3、4 或 5、或更多层肠溶包衣。例如,增加肠溶包衣的层数能够被用于制备位点特异性输送至小肠末端 (比如回肠或大肠) 的肠溶包衣组合物。

[0114] 本发明实施例所述的肠溶包衣组合物可以进一步包括内层,其位于肠溶包衣和核心之间。如本文所用,术语“内层”指薄膜或涂层,其在含有治疗活性成分核心和围绕核心的肠溶包衣之间作为物理屏障,从而防止肠溶包衣与核心接触。内层在肠溶包衣之前先覆盖在核心上。内层的功能包括:保护核心组分,由此使得核心组分和肠溶包衣组分之间没有不良反应。例如,肠溶层可以是酸性的,并且直接与核心接触,然而酸性的肠溶层导致治疗活性组分不稳定或分解,产生杂质等。内层还具有在核心上提供一层平滑基底的作用,以便进一步施涂肠溶涂层。适用于内层的材料的例子包括 (但不限于):纤维素聚合物,如甲基纤维素、乙基纤维素等;丙烯酸,如甲基丙烯酸酯和甲基丙烯酸甲酯等的均聚物或共聚物;以及乙烯基聚合物,如聚乙烯醇等。内层还可以进一步包括添加剂,如增塑剂、润滑剂、染料、着色剂、消泡剂、乳化剂、表面活性剂等。

[0115] 本发明所述的肠溶包衣组合物可配制成口服给药。根据本发明的实施方式,肠溶包衣组合物的核心较佳地为固体,比如,片剂、胶囊、丸剂、液体凝胶、颗粒剂、粉剂等。较佳地,核心为片剂的形式。为了制备肠溶包衣组合物的核心部分,用常规的药剂混合技术将甘油磷酸盐与药学上可接受的载体进行混合。

[0116] 基于本发明的公开内容,核心可以包括任何药学上可接受的载体。例如,药学上可接受的载体可以包括一种或多种赋形剂,比如,粘结剂、悬浮剂、乳化剂、润湿剂、润滑剂、调味剂、甜味剂、防腐剂、染料以及涂层。具体地,对于配制成固体 (例如,片剂、胶囊等) 核心的合适载体和添加剂,包括淀粉、糖、稀释剂、颗粒剂、润滑剂、粘结剂、崩解剂等。

[0117] 本发明所用的肠溶包衣组合物的核心可以包括治疗有效量的甘油磷酸盐, 其为核心总重的 10-100%, 较佳地, 50-100%, 更佳地, 75-99.9%。甘油磷酸盐的量取决于甘油磷酸盐的特定阳离子。例如, 如果使用甘油磷酸钙, 核心最好含有以核心的总重计大概 99.625% 的甘油磷酸钙, 以及 0.375% 重量的载体, 并且较佳地, 仅含有以核心的总重计 0.375% 重量的硬脂酸镁且无其他载体。

[0118] 根据本发明的实施方式, 肠溶包衣组合物的核心可以进一步包括 NSAID。核心可以包括甘油磷酸盐, 其重量是在甘油磷酸盐和 NSAID 的混合物中 NSAID 重量的约 10-1000%, 较佳地, 30-70%, 更佳地, 40-60%, 最佳地, 为核心中 NSAID 重量的 50%。在一个优选的实施方式中, NSAID 为布洛芬。

[0119] 根据本发明的实施方式, 本发明所述的肠溶包衣组合物可以进一步包括控释剂, 其存在于至少一个核心或肠溶层中。如本文所用, 术语“控释剂”指一种化合物或添加剂, 其能够控制组合物中治疗活性成分的释放速度, 或导致特定的延迟, 从而控制治疗活性成分被施用了该组合物的个体所利用的位置。控释剂的例子包括缓释制剂(用于缓慢释放或延迟释放)、立即释放剂(用于立即释放或分散)以及缓释剂(用于推迟释放)。

[0120] 如本文所用, 术语“缓慢释放”和“延迟释放”指治疗活性组分从药物组合物中的释放经历了一个延迟阶段。如本文所用, 术语“立即释放”指在被施用于个体的某部位后, 治疗活性组分从药物组合物中立即或短时间内释放或分解。立即释放也可以指本发明所述的肠溶包衣组合物的肠溶层已经开始溶解后, 治疗活性组分直接分散。如本文所用, 术语“推迟释放”指, 在将组合物施用于个体后或在给该个体施用该组合物后过了特定一段时间后, 只有在组合物到达该个体的预期的位置后, 该治疗活性组分才从药物组合物中释放出来, 从而实现定点释放, 即在所需位置释放。

[0121] 较佳地, 本发明所述的组合物包括控释剂, 其为缓释剂。

[0122] 如本文所用, 术语“缓释剂”指一种化合物或添加剂, 其能够控制治疗活性成分从组合物中释放出来, 由此使得, 释放的发生为位点特异性, 或释放在所需的位置发生。缓释剂是这样一种类型, 一旦进入预期的位置, 它基本上能够同时将所有的治疗活性成分都释放出来, 或它只有在已经过了个体施用该组合物后的一段特定时间后, 才会释放治疗活性成分, 由此使得组合物能够到达所需位置, 并且治疗活性成分也输送到所需位置, 即具有位点特异性。根据本发明的实施方式, 缓释剂可以存在于核心的任何部分, 或作为肠溶层的一部分。较佳地, 缓释剂作为肠溶层的一部分而存在。

[0123] 根据本发明的实施方式, 肠溶包衣组合物进一步还包括缓释剂, 只有当组合物至少已经进入到小肠或大肠时(更佳地只有当组合物已经进入小肠的十二指肠和空肠的交界处), 甘油磷酸盐才能从核心中释放出来。较佳地, 甘油磷酸盐从组合物核心中的释放, 发生于受控的 pH 范围内, 所述 pH 至少 > 5.0, 比如, 5.5、6.0、6.5、7.0、7.5 或 8.0 或更大, 并且较佳地发生于 pH 至少为 7.0 或更大。

[0124] 存在于本发明所述组合物中的缓释剂的量取决于所需的甘油磷酸盐释放曲线。例如, 缓释剂存在的重量百分比可以为 10-90%, 以组合物的总重计, 比如, 10%、20%、30%、40%、50%、60%、70%、80% 或 90%。当缓释剂具有特别耐 pH 的释放曲线时(即, 直到高 pH 才溶解, 比如至少 pH 为 7.0), 缓释剂可以以更小的重量百分比存在, 以组合物的总重计, 比如, 10%、20%、30%、40% 或 50%。

[0125] 本发明所用的合适的缓释剂的例子包括（但不限于）：凝胶、蜡、脂肪、乳化剂、脂肪和乳化剂的组合、聚合物、淀粉、纤维素聚合物等，及其组合。可以用于提供缓释剂的蜡和蜡质材料的例子包括巴西棕榈蜡、鲸蜡、小烛树蜡、可可脂、十八醇十六醇混合物、蜂蜡、部分氢化植物油、角苷脂、石蜡、肉豆寇醇、硬脂醇、鲸蜡醇、和硬脂酸。

[0126] 较佳地，缓释剂包括控速的聚合物。如本文所用，术语“控速的聚合物”指一种聚合物，其控制治疗活性成分从组合物中释放的速度，由此使得，直到组合物已经进入所需的位置（即所需的输送位置）后，治疗活性成分才会释放出来。控速的聚合物的例子包括纤维素聚合物，如羟丙基甲基纤维素（HPMC）、羟乙基纤维素（HEC）、羟丙纤维素（HPC）、羟甲基化纤维素（CMC）、及其混合物。根据本发明的实施方式，缓释剂所用的纤维素聚合物的分子量可以变化，从而优化甘油磷酸盐从核心中释放出来的速度，以实现所需的释放效果。

[0127] 下列为几个可以商业获得的、适用于本发明的控速聚合物。其包括METHOCEL®纤维素醚和ETHOCEL®乙基纤维素聚合物，可获自Colorcon公司。

[0128] 根据本发明一个优选的实施方式，控速的聚合物是pH敏感型聚合物，其存在于本发明所述的组合物中，作为肠溶包衣的一部分。在本实施方式中，肠溶包衣自身为组合物提供了缓释特性，由此使得直到组合物到达肠道的某个位置，且在某个pH条件下，对pH敏感的聚合物溶解、瓦解或分解时，肠溶包衣才会溶解，甘油磷酸盐才会从核心中释放出来。

[0129] 用于形成肠溶包衣组合物的方法，尤其是形成组合物为适合口服的固体形式的肠溶包衣组合物的方法，是本领域中已知。用于本发明方法的肠溶包衣组合物，可以这样生产：在核心上施涂肠溶包衣的溶液或悬浮液，所述核心含有治疗有效量的甘油磷酸盐，其中核心较佳地以固体口服剂型存在，比如片剂、胶囊、丸剂、液体凝胶、颗粒剂、粉剂等，更佳地，为片剂。

[0130] 根据本发明的实施方式，首先制备用于施涂于核心上的涂层配制物。如本文所用，术语“涂层配制物”指处于悬浮液/分散液或溶液的形式的水本发明所述的肠溶包衣。因此，涂层配制物包括肠溶包衣的所有组分，其或者悬浮，或者溶解在溶剂（有机溶剂或水）中。涂层配制物可以是水性悬浮液，可以是水性分散液，或者可以是有机溶液。

[0131] 以水性悬浮液或分散液形式存在的涂层配制物，可以将聚合物材料以及任何添加剂（润滑剂、增塑剂、缓释剂等）稀释或悬浮于水性溶剂（较佳地，水）而予以制备。以溶液形式存在的涂层配制物，可以将聚合物材料和任何添加剂分散或溶解于有机溶剂中而予以制备。可使用的合适的溶剂例子包括丙酮、甲醇、乙醇、异丙醇、乙酸乙酯、二氯甲烷、或其混合物等。较佳地，可将增塑剂添加到涂层配制物中，不管其以水悬浮液/分散液，还是以溶液的形式存在。

[0132] 在本发明中，可以用本领域已知的方法合成悬浮液和溶液，包括（但不限于）：混合、共混、搅拌等。

[0133] 涂层配方之后涂在核心上，得到了本发明所述的肠溶包衣组合物。任何本领域已知的将涂层配方涂到核心上，以得到本发明所述的肠溶包衣组合物的方法均可以使用。典型的将涂层配方涂到核心上的方法包括喷涂方法，比如流化床方法、喷雾干燥和侧排气涂布盘包衣（pan coating）方法。喷涂方法所用设备的例子包括流化床包衣机、离心流化床包衣机、盘式涂布机等。一般来说，上述方法涉及涂布涂层配制物（如上所述），其中用喷嘴将涂层配制物喷到核心上，之后烘干以蒸发最初存在于涂层配制物中的溶剂。烘干步骤中

的温度应当在所使用的特定肠溶包衣的制造商所建议的温度范围下进行。

[0134] 根据本发明的实施方式,涂层配方可以在核心上涂一次,也可以在核心上涂不止一次,以获得有一或多层肠溶层的肠溶包衣组合物。在某个实施方式中,涂不止一层的肠溶层是有利的,其中,每层包含一个不同的聚合物材料,或添加剂组分,以优化来自于肠溶包衣组合物中的甘油磷酸盐的溶解特性或释放特征。

[0135] 内层还可以在涂肠溶层之前就涂到核心上。可以用本领域已知的技术将内层涂到口服剂型的固体药物组合物中。该技术包括将内层喷到颗粒上,比如片剂、胶囊等,放置旋转盘上,用流化床包衣设备等进行涂层。

[0136] 甘油磷酸盐从肠溶包衣组合物的核心中释放曲线会受到各种不同参数的影响,其中包括肠溶层的厚度、涂层中的添加剂、甘油磷酸盐的溶解度以及聚合物材料的 pH 敏感性。本领域的普通技术人员能够轻易地优化这些参数,以获得用于本发明方法的、具有所需的溶解和释放效果的肠溶包衣组合物。

[0137] 使用肠溶包衣组合物的治疗方法

[0138] 基于本发明公开内容,包括治疗有效量的甘油磷酸盐(较佳地,甘油磷酸钙)的肠溶包衣组合物,可以用于治疗、减轻、缓和或预防个体的肠损伤或其症状。

[0139] 根据本发明的实施方式,待治疗或预防的肠损伤,可以是被 NSAID(较佳地,布洛芬)加重、可被运动诱发、或由运动诱发且因摄入 NSAID 而进一步加重的肠损伤。较佳地,待治疗或预防的肠损伤是由 NSAID 加重的肠损伤。例如,本发明所述的肠溶包衣组合物可用于以下方法:治疗或预防个体由 NSAID 加重的肠损伤或其症状,治疗或预防个体运动诱发的肠损伤或其症状,治疗或预防个体由运动诱发且由 NSAID 加重的肠损伤或其症状。

[0140] 较佳地,肠溶包衣组合物以口服的形式施用于个体。较佳地,甘油磷酸盐是甘油磷酸钙。

[0141] 在一个特别优选的实施方式中,本发明提供了一种治疗或预防肠损伤或其症状的方法,包括在个体中由运动诱发、由 NSAID 加重的类型,其中所述个体摄入了 NSAID,所述方法包括:以口服的形式向个体施用本发明所述的肠溶包衣组合物,其中,存在于组合物核心中的甘油磷酸盐是甘油磷酸钙。在一个优选的实施方式中,NSAID 为布洛芬。

[0142] 在另一个特别优选实施方式中,所述方法包括向个体施用肠溶包衣组合物,所述组合物含有核心,所述核心含有有效量的甘油磷酸盐(较佳地,甘油磷酸钙)和有效量的 NSAID(较佳地,布洛芬),由此使得,NSAID 和甘油磷酸钙作为同一组合物的一部分共同施用于个体。

[0143] 在另一个特别优选的实施方式中,肠溶包衣组合物进一步包括了缓释剂。

[0144] 基于本发明的公开内容,包括治疗有效量的甘油磷酸盐(较佳地,甘油磷酸钙)的肠溶包衣组合物,可用于以下方法:提高需要个体的紧密连接的完整性,以及治疗或预防个体紧密连接部功能障碍相关的疾病(如前所述)。

[0145] 据信,甘油磷酸盐的功能性基团是磷酸甘油成分。尽管钙是甘油磷酸盐优选的阳离子,然而,钙与甘油磷酸盐成分并不是甘油磷酸盐发挥功能必需一起存在的,一些其他无毒的基团也是适合用于本发明的,其中包括(但不限于):甘油磷酸钠、甘油磷酸钾、和甘油磷酸镁。然而,CGP 是较佳的甘油磷酸盐,因为 CGP 中存在松散结合的钙离子,其很容易在遇到湿时立即从甘油磷酸基团中脱离,导致在水环境能很快提供两个键,尽管与已经存在的

钙相比,钙离子的量很少,然而它仍然具有增强甘油磷酸盐对 S1P 的形成和存续性的影响。另外,对于正常 E- 钙粘蛋白的功能而言,任何量的钙都是有益的,“钙粘素”这个名字来源于“钙依赖性粘附”。应强调的是,然而,CGP 的效果并不是添加了钙而得到的,因为培养基里早已含有 1mM 的钙,而效果是在 1 μ M GCP 时表现的。

[0146] 本发明没有披露的 CGP 和其他甘油磷酸盐的其他用途,在其他各种不同专利中已有描述,包括未审定和已授权的。例如,专利号为 No. 5,869,119 的美国专利描述了用 CGP 增加酸性食物和饮料的 pH,以减少心痛和胃肠痛,并且美国专利号为 7,402,323 的专利描述了 CGP 在细胞修复中的有益效果。尽管不希望束缚于理论,据信 CGP 在各种应用中的效果是通过钙离子和存在于 CGP 分子中的甘油磷酸盐基团而协同地实现。根据本发明,在预防和 / 或治疗运动诱发的肠损伤和运动诱发且因摄入 NSAID 加重的肠损伤中,在改善肠细胞之间的紧密连接部而言,在其他益处之中,CGP 可能是根本性的。基于此原因,CGP 在增强紧密连接部完整性和预防或治疗紧密连接部功能障碍相关疾病方面是有效的。

[0147] 通过结合下列的不起限定作用的实施例,将更容易理解本发明,但是本领域技术人员应理解,这些实施例只是为了说明本发明,而本发明会由其后的权利要求更全面地进行描述。

实施例

[0148] 用 Caco - 2 细胞进行实验,Caco - 2 细胞是一株来源于结直肠癌的、异质的人上皮性结直肠腺癌细胞,它在可渗透的细胞小室装置中生长。尽管来源不同,在培养中,一旦进行长期培养,这些细胞就表现出小肠的肠细胞的特点 (C. Jumarie 等人 . J. Cell. Physiol. 149:24-33, (1991))。它们广泛用作小肠中的跨上皮运输的模型,并且被认为是腹腔疾病的模型 (G. Iacomino 等人 . J Agric Food Chem. 6 ;61(5):1088-962013(2013) ; I. Caputo 等人 . PLoS One. 7(9):e45209(2012) ;T. Rauhavirta 等人 . J Clin Immunol. ; 33(1):134-42(2013))。

[0149] (1) 跨上皮电阻的测定 :在运输性上皮细胞中,紧密连接部的紧密性可以通过测量跨膜电阻即跨上皮电阻 (TEER) 来评估。TEER 测量离子通过细胞 (跨细胞) 和在细胞之间 (细胞旁路) 的运动。在非常紧密的单层细胞中,运输几乎全部是跨细胞的,因此电阻很高。细胞在渗透性屏障 (例如,细胞小室系统) 里生长,并且用专为该目的设计的欧姆计 (例如, EVOM2 上皮伏特欧姆计) 测量跨膜电阻。在培养 3-4 周时,TEER 可在 700-800 Ω / cm^2 的范围。

[0150] (2) 甘露醇通量 :借助测量甘露醇 (一种多羟基醇) 通过细胞单层的通量,可验证 TEER 的结果。甘露醇通量是完全通过细胞旁路路径的,因此它们的运动与紧密连接部的紧密性呈反比。既可以从顶面到底侧面的方向进行测定,也可以从底侧面到顶面的方向进行测定。通量率被用于计算单层的渗透系数。甘露醇的渗透系数可能与 TEER 反向相关。

[0151] (3) E- 钙粘蛋白的表达 :E- 钙粘蛋白是紧密连接部的主要蛋白。用市售的抗体进行 Western 印迹实验,从而测得均质 Caco - 2 细胞中 E- 钙粘蛋白的水平。因为钙粘蛋白有可能作为蛋白表达,但是从细胞膜上被掩蔽,因此表面的 E- 钙粘蛋白还可以用流式细胞术或荧光显微方法进行测定。计算出表面上的 E - 钙粘蛋白与总 E- 钙粘蛋白的比例。在 CGP 存在或不存在的条件下,测量在 Caco-2 细胞中 E- 钙粘蛋白的表达水平。基于本发明

的公开内容,采用本领域技术人员已知的方法,可以测量,在 CGP 存在或不存在的条件下,Caco-2 细胞中其他的紧密连接部蛋白的表达水平。

[0152] (4) 1- 磷酸 - 鞘氨醇 :甘油磷酸钙具有作为磷酸酶抑制剂的活性。据信,CGP 通过抑制去磷酸化作用,从而提高了 S1P 的浓度。用商业化的 ELISA 试剂盒 (例如,Echelon 公司的商品目录 #K-1900),测量在 Caco-2 细胞介质中的 S1P 的浓度。在 CGP 存在和不存在的条件下,测量在 Caco-2 细胞介质中的 S1P 的浓度。

[0153] 实验条件

[0154] 在存在或不存在浓度逐渐增加的 CGP 的条件下,获得在未受刺激的细胞中每个参数的基线值。TEER 发展的时间过程以及 CGP 的适宜浓度,都用实验来确定。在单层汇合后,Caco-2 细胞中 TEER 的发展需要 18-24 天,而 CGP 的浓度预期为 1 μ M — 1mM。甘露醇通量和 TEER 数据被用于区分跨细胞的和细胞旁路的渗透性。在基线上,与跨细胞的渗透性相比,细胞旁路的渗透性可以很低,这反映出紧密连接部的“紧密性”。

[0155] 在存在和不存在 CGP 的条件下,针对每个参数,对低氧对跨上皮渗透性的影响进行测定。在含氧量正常的条件下,细胞在细胞小室中生长至汇合,然后被转移到低氧室 (95% N₂/5% CO₂),培养基更换为经 95% N₂/5% CO₂ 吹洗且不含葡萄糖的培养基。在这些条件下 (该条件可以模拟局部缺血),剩余氧的分压不到 25mm Hg (6%)。对照孔只含有培养基,剩余的孔含有浓度逐渐增加的甘油磷酸钙。TEER 和甘露醇通量在缺氧室中测量。用缺氧室制备的样品,在室内空气下对 E- 钙粘蛋白的表达或 S1P 进行测定。缺氧可降低 TEER 和 S1P,增加从顶面到底侧面以及从底侧面到顶面的甘露醇通量,而不改变 E- 钙粘蛋白的表达。这些影响可被 CGP 减轻。

[0156] 在存在和不存在 CGP 的条件下,针对每个参数,对外源性细胞因子的影响进行测定。缺氧和腹腔疾病的特征是免疫系统的细胞释放细胞因子。这些炎性介导因子增加了单层的渗透性 (M. Bayardo 等人 Clin Exp Immunol. ;168(1):95-104(2012))。在存在或不存在浓度不断增加的 CGP 条件下,细胞在细胞小室装置中生长直至汇合,然后用细胞因子 TNF α (肿瘤坏死因子 α)、IL β (白介素 β) 以及 IF γ (干扰素 γ) 的混合物 (“细胞因子混合物”) 进行侵袭。在合适的时间点,测量从顶面到底侧面的甘露醇通量。在不同的实验中,对 TEER、E- 钙粘蛋白的表达和培养介质中 S1P 的浓度进行测定。另外,对 iNOS 的表达进行测定,以便验证细胞因子处理会导致炎性反应。外源的细胞因子可以降低 TEER 增加甘露醇通量。

[0157] 针对每个参数,对 α - 醇溶蛋白的影响进行测定。α - 醇溶蛋白是一种冒犯性蛋白,在易感病人中引发腹腔症状。胃蛋白酶和胰蛋白酶剪切 α - 醇溶蛋白形成多种肽,这些肽可能是肠病的直接原因。文献 (G. Iacomino 等人, J Agric Food Chem. 6 ; 61(5):1088-962013(2013)) 建议,由 α - 醇溶蛋白的氨基酸 31-55 构成的肽可能是一种候选肽。选出最可能的候选肽 (与其他研究者一致),并且在如表 1 所示的这些实验条件下对每个参数进行测定。

[0158] 表 1

[0159]

肽 31-55	CGP	细胞因子
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存在	不存在	不存在
存在	存在	不存在
存在	存在	存在

[0160] 肽可能会增加渗透性,并且可能与细胞因子有协同作用。CGP 可将渗透性回复到正常。

[0161] 甘露醇通量分析如下进行。在实验开始(时间为 0)时,细胞的培养基更换为不含葡萄糖并且不含胎牛血清的培养基,环境从 95% O₂/5% CO₂变为 1% O₂, 5% CO₂和 94% N₂。这些条件模仿了局部缺氧状态(没有食物,没有氧气)。

[0162] 实验结果和讨论

[0163] 基线:甘露醇通量。CGP 对 Caco - 2 细胞生长在细胞小室的基线甘露醇通量的影响如图 1 所示。CGP 对跨隔膜(barrier)的甘露醇通量的影响不大。然而,需注意,在每个时间点,甘露醇的渗透性在未处理细胞组中是最大的。

[0164] 基线:TEER。CGP 对基线 TEER 的影响如图 2 所示。可以看到,柠檬酸盐产生了一个小的但统计学上显著的 TEER 下降的作用,尤其是,在 2 小时是明显的。该作用可被 CGP 防止,尽管 CGP 的效果只能维持 4 个小时。

[0165] 基线:S1P。CGP 对 1- 磷酸 - 鞘氨醇在培养基中浓度的影响如图 3 所示。细胞在逐渐增加的 CGP 浓度下孵育 24 小时。取出培养基,用酶联免疫分析法(ELISA)来分析 CGP。可以看到,在基线条件下,CGP 并没有表现出对 1- 磷酸 - 鞘氨醇浓度有影响。

[0166] 基线:E- 钙粘蛋白。可以看到,CGP 可能会增加 E- 钙粘蛋白的表达,但最可能是不改变 E- 钙粘蛋白的表达。

[0167] 总体来说,这些数据显示了在基线条件下,CGP 只对跨上皮的渗透性有小的影响。

[0168] 甘露醇通量分析。可以看到,细胞对缺血条件,通过去除非必要的功能(如维持紧密连接部)而作出反应。似乎,通过添加 CGP,细胞能够在很长一段时期里都保持紧密连接部的完整性。

[0169] 具体地,表 2 和图 4 显示了施用 CGP 后,在 2 和 4 小时,甘露醇穿透量的增加的百分比。甘露醇的运动与紧密连接部的紧密性成反比。可以看到,紧密连接部紧密性幅度的增加(或甘露醇通量的减小),与培养基中的 CGP 浓度成比例。尽管有可能细胞只是用磷酸甘油替换葡萄糖,但这是非常不可能的,因为即使在 0.000001 摩尔 CGP 下依然有效果。常规的葡萄糖的浓度为 10mM 左右,这比最高的 CGP 浓度要高 10 倍,比最低的 CGP 浓度高 10000 倍。因此,表 2 和图 4 的结果表明,给细胞施用 CGP,能够长时间保持紧密连接部的完整性。

[0170] 表 2

[0171]

CGP 浓度 (M)	每分钟分解的量	
	2 小时	4 小时
0	188.6054	2362.6050
0.001	23.10722	150.2845
0.0001	50.46854	188.7996
0.00001	67.95316	195.9237
0.000001	77.03592	275.0632

[0172] 缺氧条件下的经上皮渗透性

[0173] 缺氧 / 缺血是减少氧和营养物质供应的状态, 由此使得不能满足细胞需求。据以往观察, 大量运动 (比如长时间的骑自行车), 会诱发了一种增加经上皮渗透性的状态, 该状态会被非甾体类抗炎药 (如布洛芬) 加重。已提出如下假设: 经上皮渗透性的上升是肠缺氧 (其自身是在剧烈运动过程中血从肠道被分流所导致的结果) 的结果; 并且, 渗透性可通过 CGP 治疗而缓解。该假设如下进行验证: 将 Caco-2 细胞置于缺氧室, 并且测定从顶面到底侧面的甘露醇通量和 TEER。

[0174] 甘露醇通量。图 5 显示了在缺氧过程中 CGP 对甘露醇通量的影响。为了研究, $[^{14}\text{C}]$ 甘露醇被加入到细胞小室的顶面室, 而采样来自于底侧面室。CGP 以浓度依赖的方式, 显著减弱了缺氧所诱导的甘露醇通量。该效果具有时间依赖性, 因为它在 4 小时内减弱, 并在 5 小时消失。

[0175] TEER。图 6 显示了在缺氧过程中 CGP 对 TEER 的影响。为了研究, 细胞在细胞小室中生长直至汇合, 这时测定的 TEER 值 $>600\text{m}\Omega/\text{cm}^2$ 。由于基线 TEER 有某些可变性, 因此所有的数据以基线 TEER 的百分比进行表示。缺氧 1 小时后, 对照细胞中的 TEER 显著下降。在整个实验过程中, 对照 TEER 继续下降。然而, CGP 以浓度依赖的方式保护了 TEER。对甘露醇来说, CGP 的影响随着时间下降, 因此, 在 5 小时不再有可分辨的效果。

[0176] S1P 和 E-钙粘蛋白 (理论性实施例)

[0177] 已假定, CGP 的效果是由 S1P 介导。该假设如下进行验证: 在存在或不存在 CGP 的情况下, 将细胞置于缺氧室。在 2 和 4 小时, 取出培养基样品, 分析 S-1P 和 E-钙粘蛋白 (主要的紧密连接蛋白)。预期的是, 在经 CGP 处理的细胞中, S-1P 会更多, 而 E-钙粘蛋白没有变化。这些结果表明, 经上皮细胞完整性的保持是通过 S1P 介导的, 而不是通过 E-钙粘蛋白介导的。

[0178] 在细胞因子刺激后的经上皮渗透性

[0179] 细胞因子是一类在细胞-细胞信号传导中至关重要的小蛋白。有许多类型和功能的细胞因子。有 3 种细胞因子, 即为 $\text{TNF}\alpha$ (肿瘤坏死因子 α)、 $\text{IL}\beta$ (白介素 β) 以及 $\text{IF}\gamma$ (干扰素 γ), 涉及炎症期间的细胞信号传导。因此, 用 $\text{TNF}\alpha$ 、 $\text{IF}\gamma$ 和 $\text{IL}\beta$ 的组合来处理细胞, 可模仿炎症应答早期。炎症与本研究相关, 有二个原因。第一, 介导免疫应答的中性粒细胞和其他细胞被募集到了缺血组织。第二, 腹腔疾病的肠损伤取决于炎症。已假定, 细胞因子的刺激会增加 Caco-2 细胞的经上皮渗透性, 并且该效应可通过同时用 CGP

处理而减少。

[0180] 甘露醇通量。如图 7 所示,单独使用细胞因子混合物,以依赖时间的方式增加了经上皮的甘露醇渗透性,并且 CGP 以浓度依赖的方式推迟了渗透性的增加。令人感兴趣的是,在这些实验里,对照甘露醇渗透性显著高于经 CGP/ 细胞因子混合物处理的细胞的渗透性。这与基线研究形成对比,在基线研究中,单用 CGP 似乎对甘露醇通量的影响很小(如果有的话)。

[0181] TEER。细胞因子混合物 /TEER 实验的结果很不同,并且是出乎意料的。如图 8 所示。很明显,细胞因子混合物直到接触 8 小时才开始减少 TEER。在 10 小时时,所有的 CGP 处理组,除了 1 μ M 处理组具有显著高于单用细胞因子混合物的 TEER 之外,其余处理组与对照组无异。24 小时,所有 CGP 的效果消失了。这些结果与甘露醇通量形成明显对比,可能意味着甘露醇通量和 TEER 的维持有不同分子机理。这些结果表明,在 8 小时和 10 小时之间时间点的额外实验是有帮助的。

[0182] S1P 和 E- 钙粘蛋白。(理论性实施例)

[0183] 已假设,CGP 的效果是由 S1P 介导。该假设如下进行验证:在存在或不存在 CGP 的条件下,用细胞因子混合物处理细胞。在 2 小时和 4 小时,取出培养基样品,并对 S1P 和 E- 钙粘蛋白进行分析(主要的紧密连接蛋白)。

[0184] α - 醇溶蛋白的影响(理论性实施例)

[0185] α - 醇溶蛋白的肽片段 31-55 是冒犯性(offending)片段,它在易感病人中引发腹腔症状。甘露醇通量和 TEER 在下述实验设定下进行测定:

[0186] 1. 仅用肽。

[0187] 2. 肽 +1mM CGP

[0188] 3. 肽 + 细胞因子混合物

[0189] 4. 肽 + 细胞因子混合物 +1mM CGP

[0190] 在 TEER 实验的最后,分析培养基中的 S1P 和 E- 钙粘蛋白,并且对细胞本身进行 E - 钙粘蛋白分析。

[0191] 应理解,本领域技术人员可以在不背离宽广的本发明概念的情况下,对上述实施例进行改动。因此,应理解,本发明并不受所公开的这些具体实施例的限制,而应覆盖落于由所附权利要求限定的本发明的主旨和范围之内的这些改动形式。

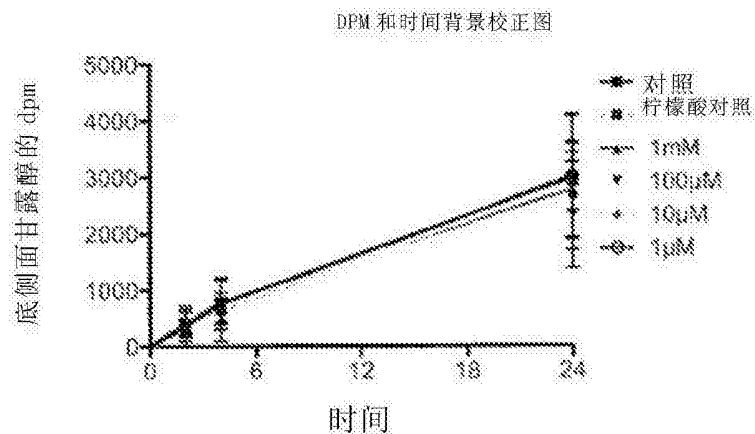


图1 在有和没有浓度增加的 CGP 存在下，基线甘露醇通量与时间的关系图

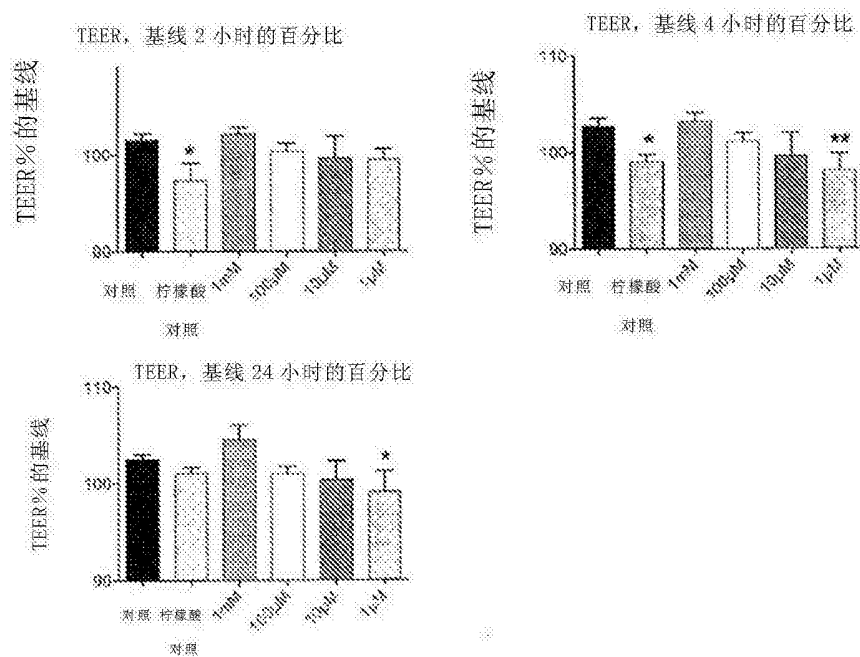


图2 在有和没有浓度增加的 CGP 存在下，CGP 对跨上皮电阻 (TEER) 的影响

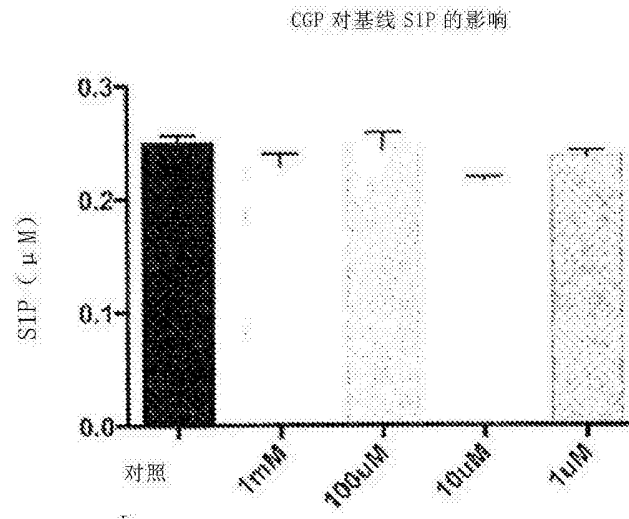
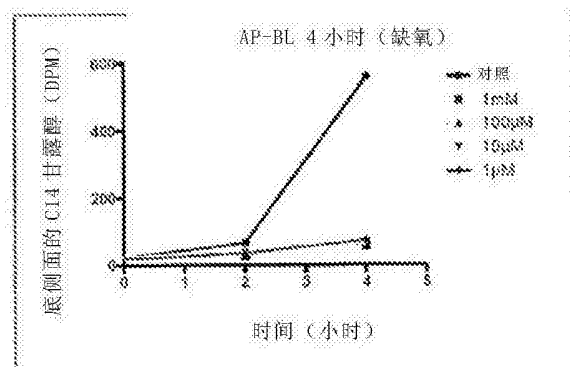


图 3 CGP 对 Caco-2 细胞中鞘氨醇 1-磷酸基线产量的影响



缺氧条件：1%氧气，葡萄糖和无血清的培养基。

C14 甘露醇以 10 μM 浓度加入。

对照没有 CGP，含有甘露醇。

图 4 向 Caco-2 细胞施用甘油磷酸钙(CGP) 4 小时后，细胞内甘露醇渗透的增加

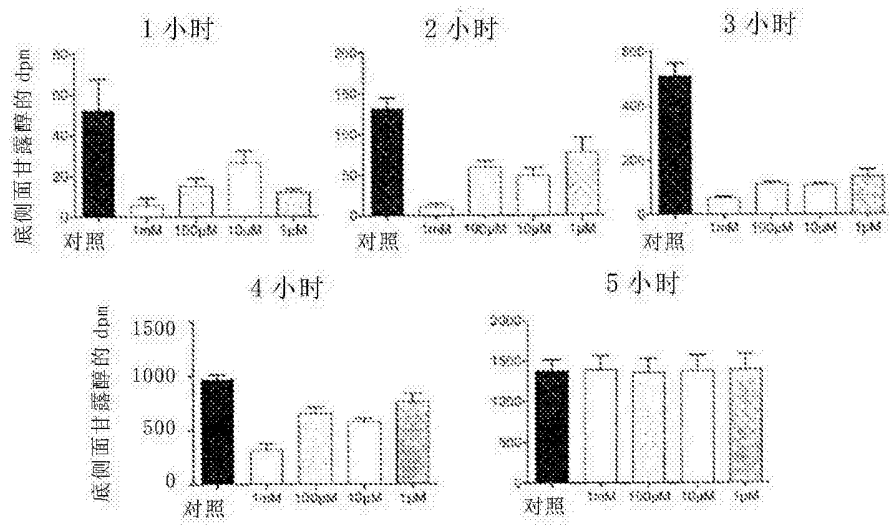


图 5 缺氧时跨上皮甘露醇通量图

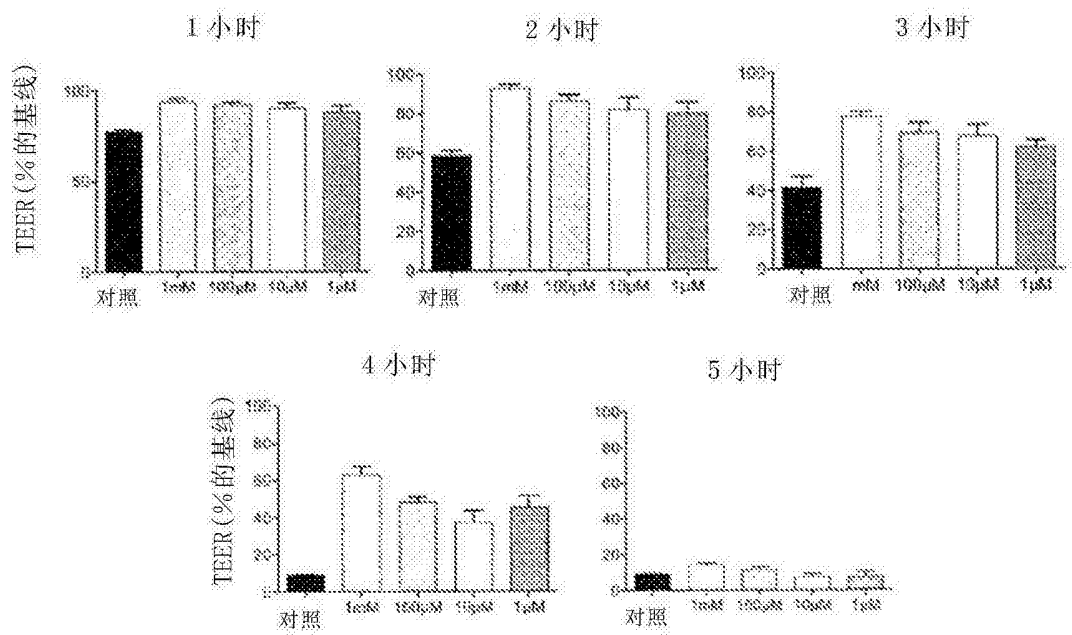


图 6 缺氧时 TEER 图

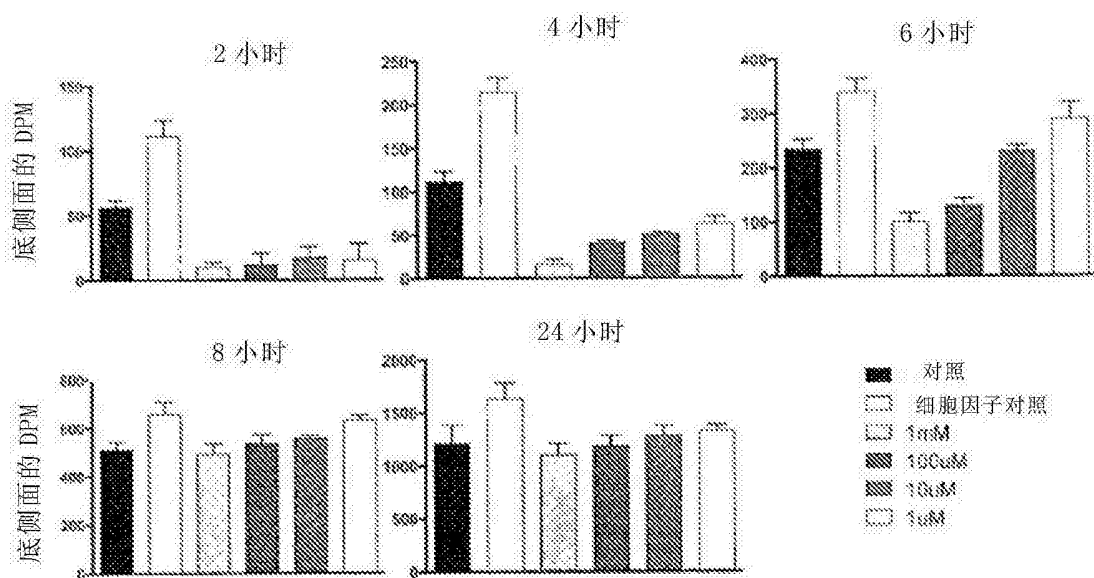


图 7 CGP 对细胞因子诱导的跨上皮甘露醇通量的影响

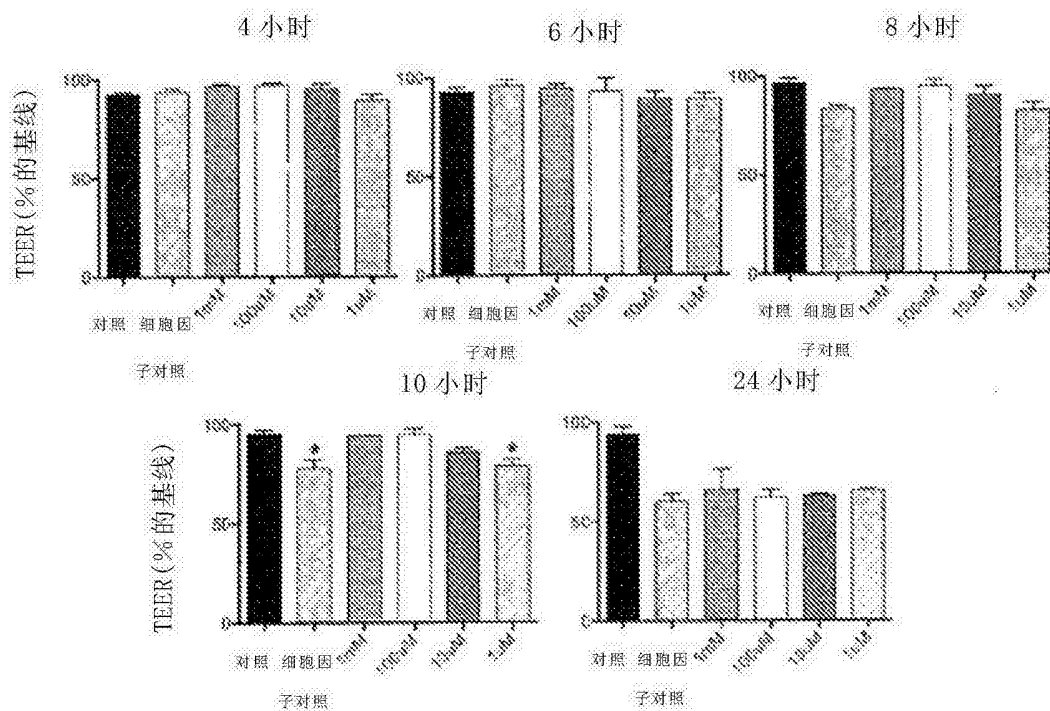


图 8 细胞因子混合物((TNF- α)、(IFN- γ)和(IL- β))处理后, CGP 对跨上皮电阻的影响。