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(54) Title: ORAL ADMINISTRATION OF DEFENSINS TO TREAT INTESTINAL DISEASES

(57) Abstract: The present invention relates, generally, to oral administration of defensins for the treatment of intestinal diseases such as ulcerative colitis and Crohn's disease. The defensins are preferably human defensins, and are more preferably human α -defensins. The defensins may be provided in a dosage form that is suitable for oral administration to a patient suffering from intestinal disease.



WO 2007/081486 A2

Oral Administration of Defensins to Treat Intestinal Diseases

Related Application Data

[0001] This application claims priority from U.S. Provisional Application No. 60/750,403, which was filed on December 15, 2005, the contents of which are incorporated herein by reference.

Background of the Invention:1. Field of the Invention

[0002] The present invention relates to the oral administration of defensins to treat intestinal diseases such as ulcerative colitis or Crohn's disease.

2. Description of the Related Art

[0003] Inflammatory bowel disease (IBD) is a chronic inflammation of the intestine. On the basis of its clinical features and histopathology it is often grouped into two major entities, ulcerative colitis and Crohn's disease. In both forms of IBD, intestinal microbes are thought to trigger the disease in genetically susceptible individuals. It is estimated that about one million Americans suffer from IBD, and in industrialized countries, it is estimated that Crohn's disease affects about 1 in 500 individuals. Unfortunately, available therapies are generally regarded as inadequate.

[0004] The exact etiology of IBD is still enigmatic. The causes are complex and likely involve the interaction between genes/host factors and environmental factors of the gut. The current hypothesis regarding pathogenesis is that disease development results from a breakdown of intestinal mucosa homeostasis maintained by host immunity and commensal flora. A disturbed antimicrobial defense, including that provided by Paneth cells of the small intestine, seems to be a critical factor in the pathogenesis of ileal Crohn's disease.

[0005] Much of the recent evidences, based on genetic, molecular, biochemical, and transgenic studies, place human α -defensins expressed in Paneth cells at the center of emerging hypotheses for ileal Crohn's disease. Virtually all species of vertebrates and invertebrates produce peptides that have broad-spectrum antibiotic activity. A major class of antimicrobial peptides is defensins. Currently, three types of defensins are known - α , β , and θ . Humans, however, appear to produce only α - and β -defensins. To date, six α -defensins and four β -defensins have been characterized in humans with about 31 defensin genes identified (Schutte, et al., PNAS 99:2129 (2002)). While β -defensins are expressed at multiple sites in the gastrointestinal tract, α -defensins are largely expressed in the small intestine.

[0006] Mature human defensins contain between 30 and 40 amino acid residues and each defensin carries 3 disulfide bonds. Human defensins are effective broad spectrum antimicrobials, particularly in high concentration and low ionic strength. Among the human α -defensins, four are expressed in neutrophils and two are primarily expressed in Paneth cells. Paneth cells are secretory epithelial cells and the major source of antimicrobial peptides in the small intestine, including human α -defensins 5 and 6 (HD5 and HD6). Among the proteins secreted by Paneth cells, the most abundant ones are HD5 and HD6. Between the two, HD5 is more abundant and much better studied. Paneth cells, located at the base of the crypts of the small intestine, secrete antimicrobial peptides and proteins that contribute to the innate immune defense of the small intestine. Paneth cell products are thought to limit the number of commensal bacteria and impact on their composition. A lack of Paneth cell products, such as HD5 and HD6, has been shown to be associated with ileal Crohn's disease.

[0007]

There is clearly a great need in the art for compositions and methods for the oral administration of defensins. There is an especially great need in the art for effective compositions and methods for treating IBD including ulcerative colitis, and ileal Crohn's disease.

Summary of the Invention

[0008]

The present invention relates to the treatment of intestinal diseases, and preferably to the treatment of IBD (ulcerative colitis, and Crohn's disease), by orally administering defensins to a patient suffering from any of these conditions. The present invention also relates to pharmaceutical preparations comprising at least one defensin. There is an unmet need in the art for such compositions and methods.

[0009]

The present invention meets the unmet needs in the art, as well as other, by providing a method for treating intestinal diseases by oral administration of defensins.

[0010]

In some embodiments, the present invention is directed to a method for treating intestinal diseases, such as IBD, ulcerative colitis or Crohn's disease, by oral administration of at least one human defensin, preferably a human α -defensin.

[0011]

In other embodiments, the present invention is directed to a method for treating intestinal diseases, such as IBD, ulcerative colitis or Crohn's disease, by oral administration of human α -defensin 5 or 6.

[0012]

In still further embodiments, the present invention is directed to a pharmaceutical composition comprising at least one defensin formulated for oral administration.

[0013] Other novel features and advantages of the present invention will

become apparent to those skilled in the art upon examination of the following or upon learning by practice of the invention.

Detailed Description of the Preferred Embodiments

[0014] The present invention relates to methods of treating intestinal diseases by orally administering at least one defensin, and to compositions for oral administration that comprise one or more defensins. The compositions and method of the present invention may be beneficially utilized to treat subjects suffering from IBD, ulcerative colitis, and Crohn's disease.

[0015] Human defensins that are the subject to the invention include, but are not limited to, α -defensin 1 (HD1), α -defensin 2 (HD2), α -defensin 3 (HD3), α -defensin 4 (HD4), α -defensin 5 (HD5), α -defensin 6 (HD6), β -defensin 1 (HBD1), β -defensin 2 (HBD2), β -defensin 3 (HBD3), and β -defensin 4 (HBD4).

[0016] One aspect of the present invention is directed to the management of intestinal diseases, such as IBD, ulcerative colitis, or ileal Crohn's disease, with oral administration of human defensins. Preferably, treatment is with natural or recombinant human defensins, preferably α -defensins. Most preferably, the α -defensins are of the type naturally produced in Paneth cells.

[0017] Oral administration is a preferred method of administering defensins due to the ease of patient compliance. The defensins are not subject to degradation in the gut prior to providing effective treatment.

[0018] The oral formulations containing defensins according to the present invention may be administered in any dose necessary to treat the intestinal disease at issue. In one embodiment of the present invention, the oral formulation is administered in doses of from about 1 mg/patient/day to about 10 g/patient/day,

preferably 5 mg/patient/day to 5 g/patient/day, more preferably 25 mg/patient/day to

2.5 g/patient/day.

[0019] The oral formulations according to the present invention can be formulated in any manner suitable to deliver the dose. Examples include a tablet, a caplet, a hard or soft capsule, a lozenge, a cachet, a dispensable powder, granules, a suspension or solution, an elixir, a liquid, or any other form reasonably adapted for oral administration.

[0020] To help the release of defensin in small intestine, the material is preferably tableted and capsulated, and preferably enteric-coated. Enteric coating prevents a tablet or capsule from dissolving before it reaches the small intestine. Alternatively the material may be spheronized into microparticles and preferably enterically coated. Spheroids may be produced in the size range of 250 μ m to 850 μ m. Enteric coatings are known to be selectively insoluble substances that do not dissolve in the acidic environment of the stomach, but dissolve in the higher pH of the small intestine, resulting in a specific release of defensin in the small intestine.

[0021] Defensins can be further formulated together with one or more pharmaceutically acceptable excipients to produce a pharmaceutical composition. The term "excipient" herein means any substance, not itself a therapeutic agent, used as a carrier or vehicle for delivery of a therapeutic agent to a subject or added to a pharmaceutical composition to improve its handling or storage properties or to permit or facilitate formation of a dose unit of the composition into a discrete article such as a capsule or tablet suitable for oral administration. Excipients include, by way of illustration and not limitation, diluents, disintegrants, binding agents, adhesives, wetting agents, lubricants, glidants, crystallization inhibitors, surface modifying agents, substances added to mask or counteract a disagreeable taste or

odor, flavors, dyes, fragrances, and substances added to improve appearance of the composition.

[0022] Excipients employed in compositions of the invention can be solids, semi-solids, liquids or combinations thereof. Compositions of the invention containing excipients can be prepared by any known technique of pharmacy that comprises admixing an excipient with a drug or therapeutic agent.

[0023] Suitable diluents illustratively include, either individually or in combination, lactose, including anhydrous lactose and lactose monohydrate; starches, including directly compressible starch and hydrolyzed starches (e.g., Celutab™ and Emdex™); mannitol; sorbitol; xylitol; dextrose (e.g., Cerelease™ 2000) and dextrose monohydrate; dibasic calcium phosphate dihydrate; sucrose-based diluents; confectioner's sugar; monobasic calcium sulfate monohydrate; calcium sulfate dihydrate; granular calcium lactate trihydrate; dextrates; inositol; hydrolyzed cereal solids; amylose; celluloses including microcrystalline cellulose, food grade sources of α - and amorphous cellulose (e.g., Rexcel™) and powdered cellulose; calcium carbonate; glycine; bentonite; polyvinylpyrrolidone; and the like. Such diluents, if present, constitute in total about 5% to about 99%, preferably about 10% to about 85%, and more preferably about 20% to about 80%, of the total weight of the oral composition. The diluent or diluents selected preferably exhibit suitable flow properties and, where tablets are desired, compressibility.

[0024] Suitable disintegrants include, either individually or in combination, starches, including sodium starch glycolate (e.g., Explotab™ of PenWest) and pregelatinized corn starches (e.g., National™ 1551, National™ 1550, and Colocorn™ 1500), clays (e.g., Veegum™ HV), celluloses such as purified cellulose, microcrystalline cellulose, methylcellulose, carboxymethylcellulose and sodium

carboxymethylcellulose, croscarmellose sodium (e.g., Ac-Di-Sol™ of FMC),

alginates, crospovidone, and gums such as agar, guar, locust bean, karaya, pectin and tragacanth gums.

[0025] Disintegrants may be added at any suitable step during the preparation of the composition, particularly prior to granulation or during a lubrication step prior to compression. Such disintegrants, if present, constitute in total about 0.2% to about 30%, preferably about 0.2% to about 10%, and more preferably about 0.2% to about 5%, of the total weight of the composition.

[0026] Suitable binding agents and adhesives include, either individually or in combination, acacia; tragacanth; sucrose; gelatin; glucose; starches such as, but not limited to, pregelatinized starches (e.g., National™ 1511 and National™ 1500); celluloses such as, but not limited to, methylcellulose and carmellose sodium (e.g., Tylose™); alginic acid and salts of alginic acid; magnesium aluminum silicate; PEG; guar gum; polysaccharide acids; bentonites; povidone, for example povidone K-15, K-30 and K-29/32; polymethacrylates; HPMC; hydroxypropylcellulose (e.g., Klucel™); and ethylcellulose (e.g., Ethocel™). Such binding agents and/or adhesives, if present, constitute in total about 0.5% to about 25%, preferably about 0.75% to about 15%, and more preferably about 1% to about 10%, of the total weight of the composition.

[0027] Compositions of the invention optionally comprise one or more pharmaceutically acceptable wetting agents. Non-limiting examples of surfactants that can be used as wetting agents in compositions of the invention include quaternary ammonium compounds, for example benzalkonium chloride, benzethonium chloride and cetylpyridinium chloride, dioctyl sodium sulfosuccinate, polyoxyethylene alkylphenyl ethers, for example nonoxynol 9, nonoxynol 10, and

octoxynol 9, poloxamers (polyoxyethylene and polyoxypropylene block copolymers), polyoxyethylene fatty acid glycerides and oils, for example polyoxyethylene (8) caprylic/capric mono- and diglycerides (e.g., Labrasol™ of Gattefosse), polyoxyethylene (35) castor oil and polyoxyethylene (40) hydrogenated castor oil; polyoxyethylene alkyl ethers, for example polyoxyethylene (20) cetostearyl ether, polyoxyethylene fatty acid esters, for example polyoxyethylene (40) stearate, polyoxyethylene sorbitan esters, for example polysorbate 20 and polysorbate 80 (e.g., Tween™ 80 of ICI), propylene glycol fatty acid esters, for example propylene glycol laurate (e.g., Lauroglycol™ of Gattefossé), sodium lauryl sulfate, fatty acids and salts thereof, for example oleic acid, sodium oleate and triethanolamine oleate, glyceryl fatty acid esters, for example glyceryl monostearate, sorbitan esters, for example sorbitan monolaurate, sorbitan monooleate, sorbitan monopalmitate and sorbitan monostearate, tyloxapol, and mixtures thereof. Such wetting agents, if present, constitute in total about 0.25% to about 15%, preferably about 0.4% to about 10%, and more preferably about 0.5% to about 5%, of the total weight of the composition.

[0028] Compositions of the invention optionally comprise one or more pharmaceutically acceptable lubricants (including anti-adherents and/or glidants). Suitable lubricants include, either individually or in combination, glyceryl behapate (e.g., Compritol™ 888); stearic acid and salts thereof, including magnesium, calcium and sodium stearates; hydrogenated vegetable oils (e.g., Sterotex™); colloidal silica; talc; waxes; boric acid; sodium benzoate; sodium acetate; sodium fumarate; sodium chloride; DL-leucine; PEG (e.g., Carbowax™ 4000 and Carbowax™ 6000); sodium oleate; sodium lauryl sulfate; and magnesium lauryl sulfate. Such lubricants, if present, constitute in total about 0.1% to about 10%, preferably about 0.2% to about

8%, and more preferably about 0.25% to about 5%, of the total weight of the composition.

[0029] Suitable anti-adherents include talc, cornstarch, DL-leucine, sodium lauryl sulfate and metallic stearates. Talc is a preferred anti-adherent or glidant used, for example, to reduce formulation sticking to equipment surfaces and also to reduce static in the blend. Talc, if present, constitutes about 0.1% to about 10%, more preferably about 0.25% to about 5%, and still more preferably about 0.5% to about 2%, of the total weight of the composition.

[0030] Glidants can be used to promote powder flow of a solid formulation. Suitable glidants include colloidal silicon dioxide, starch, talc, tribasic calcium phosphate, powdered cellulose and magnesium trisilicate.

[0031] Other excipients such as colorants, flavors and sweeteners are known in the pharmaceutical art and can be used in compositions of the present invention. Tablets can be coated, for example with an enteric coating, or uncoated. Compositions of the invention can further comprise, for example, buffering agents.

[0032] Optionally, one or more effervescent agents can be used as disintegrants and/or to enhance organoleptic properties of compositions of the invention. When present in compositions of the invention to promote dosage form disintegration, one or more effervescent agents are preferably present in a total amount of about 30% to about 75%, and preferably about 45% to about 70%, for example about 60%, by weight of the composition.

[0033] An "effervescent agent" herein is an agent comprising one or more compounds which, acting together or individually, evolve a gas on contact with water. The gas evolved is generally oxygen or, most commonly, carbon dioxide. Preferred effervescent agents comprise an acid and a base that react in the

presence of water to generate carbon dioxide gas. Preferably, the base comprises

an alkali metal or alkaline earth metal carbonate or bicarbonate and the acid

comprises an aliphatic carboxylic acid.

[0034] Non-limiting examples of suitable bases as components of effervescent agents useful in the invention include carbonate salts (e.g., calcium carbonate), bicarbonate salts (e.g., sodium bicarbonate), sesquicarbonate salts, and mixtures thereof.

[0035] Non-limiting examples of suitable acids as components of effervescent agents useful in the invention include citric acid, tartaric acid, malic acid, fumaric acid, adipic acid, succinic acid, acid anhydrides of such acids, acid salts of such acids, and mixtures thereof.

[0036] In a preferred embodiment of the invention, where the effervescent agent comprises an acid and a base, the weight ratio of the acid to the base is about 1:100 to about 100:1, more preferably about 1:50 to about 50:1, and still more preferably about 1:10 to about 10:1. In a further preferred embodiment of the invention, where the effervescent agent comprises an acid and a base, the ratio of the acid to the base is approximately stoichiometric.

[0037] Solid dosage forms of the invention can be prepared by any suitable process, not limited to processes described herein.

[0038] An illustrative process comprises (a) a step of blending at least one defensin of the invention with one or more excipients to form a blend, and (b) a step of tableting or encapsulating the blend to form tablets or capsules respectively.

[0039] In a preferred process, solid dosage forms are prepared by a process comprising (a) a step of blending at least one defensin with one or more excipients to form a blend, (b) a step of granulating the blend to form a granulate, and (c) a step of

tableting or encapsulating the blend to form tablets or capsules respectively. Step

(b) can be accomplished by any dry or wet granulation technique known in the art, but is preferably a wet granulation step followed by a step of drying the resulting granulate prior to tableting or encapsulating.

[0040] One or more diluents, one or more disintegrants and one or more binding agents are preferably added, for example in the blending step, a wetting agent can optionally be added, for example in the granulating step, and one or more disintegrants are preferably added after granulating but before tableting or encapsulating. A lubricant is preferably added before tableting. Blending and granulating can be performed independently under low or high shear. A process is preferably selected that forms a granulate that is uniform in drug content, that readily disintegrates, that flows with sufficient ease so that weight variation can be reliably controlled during capsule filling or tableting, and that is dense enough in bulk so that a batch can be processed in the selected equipment and individual doses fit into the specified capsules or tablet dies.

[0041] In an alternative embodiment, solid dosage forms are prepared by a process that includes a spray drying step, wherein the α -defensin is suspended with one or more excipients in one or more sprayable liquids, preferably a non-aqueous sprayable liquid, and then is rapidly spray dried over a current of warm air. A granulate or spray dried powder resulting from any of the above illustrative processes can be compressed or molded to prepare tablets or encapsulated to prepare capsules. Conventional tableting and encapsulation techniques known in the art can be employed. Where coated tablets are desired, conventional coating techniques are suitable.

[0042] Defensins from any source may be used in accordance with the present invention. For example, defensins may be extracted from natural sources, chemically synthesized, or produced recombinantly from any suitable organism, such as eukaryotic or prokaryotic. Preferred recombinant hosts are yeast cells, plant cells, or bacterial cells. Recombinant production in any suitable host would be of routine skill to those in the art. See, e.g., Sambrook et al, Molecular Cloning: A Laboratory Manual, CSH Laboratory Press, 3rd Edition, 2001, hereby incorporated by reference.

[0043] It will, of course, be appreciated that the above description has been given by way of example only and that modifications in detail may be made within the scope of the present invention.

[0044] Throughout this application, various patents and publications have been cited. The disclosures of these patents and publications in their entireties are hereby incorporated by reference into this application, in order to more fully describe the state of the art to which this invention pertains.

[0045] The invention is capable of considerable modification, alteration, and equivalents in form and function, as will occur to those ordinarily skilled in the pertinent arts having the benefit of this disclosure.

[0046] While the present invention has been described for what are presently considered the preferred embodiments, the invention is not so limited. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the detailed description provided above.

1. A method for treating an intestinal disease in a patient, comprising the step of orally administering at least one defensin to a patient in an amount effective to treat an intestinal disease.
2. The method of claim 1, wherein the intestinal disease being treated is selected from the group consisting of ulcerative colitis and Crohn's disease.
3. The method of claim 1, wherein the at least one defensin is selected from the group consisting of human α -defensin 1, human α -defensin 2, human α -defensin 3, human α -defensin 4, human α -defensin 5, human α -defensin 6, human β -defensin 1, human β -defensin 2, human β -defensin 3, and human β -defensin 4.
4. The method of claim 1, wherein the at least one defensin is obtained using a technique selected from the group consisting of extraction from a natural source of defensins, chemical synthesis, and recombinant production.
5. The method of claim 1, wherein the at least one defensin is a human defensin.
6. The method of claim 5, wherein the at least one defensin is a human α -defensin.
7. The method of claim 6, wherein the human α -defensin is human α -defensin 5.

8. The method of claim 6, wherein the human α -defensin is human α -defensin 6.
9. The method of claim 1, wherein the at least one defensin is administered in an amount from about 1 mg/patient/day to about 10 g/patient/day.
10. A pharmaceutical composition comprising at least one defensin and one or more excipients formulated for oral administration.
11. The pharmaceutical composition of claim 10, wherein the at least one defensin is selected from the group consisting of human α -defensin 1, human α -defensin 2, human α -defensin 3, human α -defensin 4, human α -defensin 5, human α -defensin 6, human β -defensin 1, human β -defensin 2, human β -defensin 3, and human β -defensin 4.
12. The pharmaceutical composition of claim 10, wherein the formulation is provided in a form selected from the group consisting of a tablet, caplet, hard capsule, soft capsule, lozenge, cachet, powder, granules, suspension, solution, elixir, or liquid.