TREATMENT OF RHINITIS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application No. 61/552,508, filed October 28, 2011 and U.S. Provisional Patent Application No. 61/561,215, filed November 17, 2011. The entire disclosures of each U.S. Provisional Patent Applications Nos. 61/552,508 and 61/561,215 are incorporated herein by reference.

FIELD OF INVENTION

The invention relates to a method of treating rhinitis. The method comprises administering an effective amount of a pharmaceutical composition comprising a diketopiperazine with amino acid side chains of aspartic acid and alanine (DA-DKP). The invention also provides a pharmaceutical product comprising a DA-DKP containing composition.

BACKGROUND

Rhinitis is caused by chronic or acute inflammation of the mucous membranes of the nose due to viruses, bacteria or irritants. The inflammation results in the generation of excessive amounts of mucous, commonly producing a runny nose, nasal congestion and post-nasal drip. Rhinitis is reported to affect more than 50 million people in the United States alone.

There are several types of rhinitis, including infectious rhinitis, allergic rhinitis and nonallergic rhinitis. Infectious rhinitis is caused by a viral or bacterial infection. Types of infectious rhinitis include the common cold and sinusitis.

Allergic rhinitis affects more than 20% of people worldwide and the prevalence increases annually. Allergic rhinitis causes impaired social life, sleep, school, and work. The quality of life of patients can be altered by the severity and duration of rhinitis. Allergic rhinitis is a proinflammatory immune response to outdoor or indoor allergens, such as dust or pollen. Symptoms can occur year-round or primarily be at certain times of the year, usually in the spring, summer or fall. The Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines outlines the management of allergic rhinitis as allergen avoidance, patient education, pharmacotherapy, and allergen-specific immunotherapy. For pharmacotherapy, ARIA currently recommends intranasal, second-generation H1-antihistamines and an intranasal corticosteroid for moderate to severe persistent disease.

See Bousquet et al., *J. Allergy Clin. Immunol.*, 108(Suppl 5):S147-334 (2001) and Bousquet et al., *Allergy,* 63(Suppl. 86):8-160 (2008).

Nonallergic rhinitis is rhinitis that is not triggered by allergens or infectious agents. There is still much to be learned about nonallergic rhinitis, but it is thought that the triggers of it cause dilation of the blood vessels in the lining of the nose, which results in swelling and drainage. Types of nonallergic rhinitis include vasomotor, autonomic, hormonal, drug-induced, atrophic and gustatory rhinitis and rhinitis medicamentosa. Triggers of vasomotor rhinitis include smells, fumes, smoke, dust and temperature changes, and vasomotor rhinitis can coexist with allergic rhinitis. Rhinitis medicamentosa is a condition of rebound nasal congestion brought on by extended use of topical decongestants.

SUMMARY OF THE INVENTION

One embodiment of the invention relates to a method of treating rhinitis by administering an effective amount of a pharmaceutical composition comprising a diketopiperazine with amino acid side chains of aspartic acid and alanine (DA-DKP), to an animal in need thereof. In one aspect, the rhinitis is allergic rhinitis.

In one aspect, the effective amount of the DA-DKP in the composition is from about 100 μg to about 3000 μg per day. In another aspect, the effective amount of the DA-DKP in the composition is from about 500 μg to about 1500 μg per day.

In yet other aspects, the administration of the composition comprising DA-DKP, is commenced within 24 hours of diagnosis of rhinitis. In still other aspects, the administration of the composition comprising DA-DKP, is commenced at the appearance of one or more early signs of, or a predisposition to develop, rhinitis. One or more early signs of rhinitis can be rhinorrhea, nasal congestion, nasal itching and sneezing.

In yet other aspects of the method, the DA-DKP is in a composition prepared by removing albumin from a solution of a human serum albumin composition. For example, the step of removing can be by treating the human serum albumin composition by a separation method. Such separation methods can include ultrafiltration, sucrose gradient centrifugation, chromatography, salt precipitation, and sonication. In addition, the step of removing can be by passing the human serum albumin composition over an ultrafiltration membrane with a molecular weight cut off that retains the albumin, and the resulting filtrate contains the DA-DKP. In one aspect, the ultrafiltration membrane has a molecular weight cutoff of less than 50 kDa. In still another aspect, the ultrafiltration membrane has

a molecular weight cut off less than 40 kDa, less than 30 kDa, less than 20 kDa, less than 10 kDa, less than 5 kDa or less than 3 kDa.

In yet another aspect, the composition comprising DA-DKP is administered in combination with a second drug suitable for treating rhinitis. For example, the second drug can be selected from antihistamines, decongestants, anti-inflammatories, mast cell stabilizers, leukotriene modifiers and IgE blockers.

Another embodiment of the invention relates to a pharmaceutical product, comprising a DA-DKP containing composition formulated for administration by a route selected from inhalation, insufflation and nasal administration to the nose. In one aspect, the DA-DKP containing composition formulated for administration by inhalation is packaged in a device selected from insufflators, nebulizers, pressurized packs, squeeze bottle, a syringe, a dropper, a spray device, an atomizer device, and an aerosolizer. In one aspect, the pressurized pack can comprise a propellant selected from dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, and carbon dioxide.

In still another aspect of the pharmaceutical product, the DA-DKP containing composition formulated for administration by inhalation or insufflation comprises a powder mix of the DA-DKP containing composition and a powder base. In one aspect, the powder mix can be in a dosage form selected from capsules, cartridges, gelatin packs and blister packs. The powder mix can be delivered by a device selected from an inhalator, insufflator and metered-dose inhaler.

In yet another aspect of the pharmaceutical product, the DA-DKP containing composition formulated for nasal administration is in a form of drops or sprays. In one aspect, the DA-DKP in the DA-DKP containing composition comprises from about 0.1% (w/v) to about 10% (w/v) of the composition. The drops or sprays can be contained within an intranasal delivery system. In one aspect, the intranasal delivery system comprises an atomizing device. In one aspect, the atomizing device comprises a bottle and pump. In a preferred aspect, the pump is a metered dose pump. The metered dose pump can deliver an intranasal volume of the DA-DKP containing composition. In one aspect, the metered dose pump can deliver an intranasal volume of about 0.15 ml of the DA-DKP containing composition per pump. In still another pharmaceutical product further comprises an aqueous or non-aqueous base comprising one or more agents selected from dispersing agents, solubilizing agents, and suspending agents.

In still another aspect of the pharmaceutical product, the DA-DKP containing composition is formulated for nasal administration in a form selected from ointments, gels and creams. In one aspect, pharmaceutical product further comprises excipients selected from animal fats, vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycol, silicones, bentonites, silicic acid, talc, zinc oxide and mixtures thereof. In still another aspect, the pharmaceutical product further comprises an absorption or permeation enhancer. In yet another aspect, the pharmaceutical product further comprises a thickening agent or viscosity enhancer to increase the residence time of the DA-DKP containing composition in the nose. In still another aspect, the pharmaceutical product further comprises a pharmaceutically-acceptable carrier.

In yet other aspects of the pharmaceutical product, the DA-DKP is in a composition prepared by removing albumin from a solution of a human serum albumin composition. For example, the step of removing can be by treating the human serum albumin composition by a separation method. Such separation methods can include ultrafiltration, sucrose gradient centrifugation, chromatography, salt precipitation, and sonication. In addition, the step of removing can be by passing the human serum albumin composition over an ultrafiltration membrane with a molecular weight cut off that retains the albumin, and the resulting filtrate contains the DA-DKP. In one aspect, the ultrafiltration membrane has a molecular weight cutoff of less than 50 kDa. In still another aspect, the ultrafiltration membrane has a molecular weight cut off less than 40 kDa, less than 30 kDa, less than 20 kDa, less than 10 kDa, less than 5 kDa or less than 3 kDa.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides for a method of treating rhinitis, including but not limited to infectious rhinitis, allergic rhinitis and nonallergic rhinitis. The method comprises administering to an animal in need thereof an effective amount of a pharmaceutical composition comprising DA-DKP.

The invention also provides a pharmaceutical product formulated for administration by a route such as inhalation, insufflation or nasal administration to the nose. The composition comprises a DA-DKP containing composition.

Allergic rhinitis is a proinflammatory immune response to outdoor or indoor allergens, such as dust or pollen. Nonallergic rhinitis is rhinitis that is not triggered by allergens or infectious agents. Types of nonallergic rhinitis include but are not limited to

vasomotor, autonomic, hormonal, drug-induced, atrophic and gustatory rhinitis and rhinitis medicamentosa.

In order to treat rhinitis, the composition comprising DA-DKP is administered to an animal in need of treatment. Preferably, the animal is a mammal, such as a rabbit, goat, dog, cat, horse or human. Most preferably, the animal is a human.

Effective dosage forms, modes of administration and dosage amounts for the compounds of the invention (*i.e.,* a composition comprising DA-DKP, and preparations comprising a filtrate of DA-DKP, such as a <5000MW fraction as discussed below) may be determined empirically using the guidance provided herein. It is understood by those skilled in the art that the dosage amount will vary with the particular disease or condition to be treated, the severity of the disease or condition, the route(s) of administration, the duration of the treatment, the identity of any other drugs being administered to the animal, the age, size and species of the animal, and like factors known in the medical and veterinary arts. In general, a suitable daily dose of a compound of the present invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. However, the daily dosage will be determined by an attending physician or veterinarian within the scope of sound medical judgment. If desired, the effective daily dose may be administered as two, three, four, five, six or more sub-doses, administered separately at appropriate intervals throughout the day. Administration of the compound should be continued until an acceptable response is achieved. Such an acceptable response may be for example when the symptoms of rhinitis are reduced and/or when the symptoms of rhinitis are no longer detected by the subject.

The composition of the present invention may be a pharmaceutical solution having a DA-DKP concentration range with a lower endpoint of about 10 μM, about 20 μM, about 30 μM, about 40 μM, about 50 μM, about 60 μM, about 70 μM, about 80 μM, about 90 μM, about 100 μM, about 110 μM, about 120 μM, about 130 μM, about 140 μM, about 150 μM, about 160 μM, about 170 μM, about 180 μM, about 190 μM, about 200 μM, about 210 μM, about 220 μM, about 230 μM, about 240 μM, about 240, about 250 μM, about 260 μM, about 270 μM, about 280 μM, about 290 μM, about 300 μM, about 310, about 320 μM, about 330 μM, about 340 μM, about 350 μM, about 360 μM, about 370 μM, about 380 μM, about 390 μM, or about 400 μM. The composition of the present invention may be a pharmaceutical solution having a DA-DKP concentration range with an upper endpoint of about 600 μM, about 580 μM, about 570 μM, about 560 μM, about

550 μM, about 540 μM, about 530 μM, about 520 μM, about 510 μM, about 500 μM, about 490 μM, about 480 μM, about 470 μM, about 460 μM, about 450 μM, about 440 μM, about 430 μM, about 420 μM, about 410 μM, about 400 μM, about 390 μM, about 380 μM, about 370 μM, about 360 μM, about 350, about 340 μM, about 330 μM, about 320 μM, about 310 μM, about 300 μM, about 290 μM, about 280, about 270 μM, about 260 μM, about 250 μM, about 240 μM, about 230 μM, about 220 μM, about 210 μM, or about 200 μM.

An effective amount of DA-DKP in the composition of the present invention for treating rhinitis can be a range with a lower endpoint of about 10 μg, about 15 μg, about 20 μg, about 25 μg, about 30 μg, about 35 μg, about 40 μg, about 45 μg, about 50 μg, about 55 μg, about 60 μg, about 65 μg, about 70 μg, about 75 μg, about 80 μg, about 85 μg, about 90 μg, about 95 μg, about 100 μg, about 110 μg, about 120 μg, about 130 μg, about 140 μg, about 150 μg, about 160 μg, about 170 μg, about 180 μg, about 190 μg, about 200 μg, about 210 μg, about 220 μg, about 230 μg, about 240 μg, about 250 μg, about 260 μg, about 270 μg, about 280 μg, about 290 μg, about 300 μg, about 310 μg, about 320 μg, about 330 μg, about 340 μg, about 350 μg, about 360 μg, about 370 μg, about 380 μg, about 390 μg, about 400 μg, about 425 μg, about 450 μg, about 475 μg or about 500 μg. An effective amount of DA-DKP in the composition of the present invention for treating rhinitis can be a range with upper endpoint of about 500 μg, about 490 μg, about 480 μg, about 470 μg, about 460 μg, about 450 μg, about 440 μg, about 430 μg, about 420 μg, about 410 μg, about 400 μg, about 390 μg, about 380 μg, about 370 μg, about 360 μg, about 350 μg, about 340 μg, about 330 μg, about 320 μg, about 310 μg, about 300 μg, about 290 μg, about 280 μg, about 270 μg, about 260 μg, about 250 μg, about 240 μg, about 230 μg, about 220 μg, about 210 μg, about 200 μg, about 190 μg, about 180 μg, about 170 μg, about 160 μg, about 150 μg, about 140 μg, about 130 μg, about 120 μg, about 110 μg, about 100 μg, about 90 μg, about 80 μg, about 70 μg, about 60 μg, about 50 μg, about 40 μg, about 30 μg, or about 20 μg.

Additionally, when the DA-DKP containing composition is administered as a spray composition or formulation and/or as drop composition or formulation, the effective dosage amounts can be a range with a lower end point of about 0.01% (weight/volume (w/v)), about 0.02% (w/v), about 0.03% (w/v), about 0.04% (w/v), about 0.05% (w/v), about 0.06% (w/v), about 0.07% (w/v), about 0.08% (w/v), about 0.09% (w/v), about 0.10% (w/v), about 0.11% (w/v), about 0.12% (w/v), about 0.13% (w/v), about 0.14%

(w/v), about 0.15% (w/v), about 0.16% (w/v), about 0.17% (w/v), about 0.18% (w/v), about 0.19% (w/v), about 0.20% (w/v), about 0.25% (w/v), about 0.30% (w/v), about 0.35% (w/v), about 0.40% (w/v), about 0.45% (w/v), about 0.50% (w/v). Most preferably about 0.1% (w/v). Also, when the DA-DKP containing composition is administered as a spray composition or formulation and/or as drop composition or formulation, the effective dosage amounts can be a range with a upper end point about 10.0% (w/v), about 9.0% (w/v), about 8.0% (w/v), about 7.0% (w/v), about 6.0% (w/v), about 5.0% (w/v), about 4.0% (w/v), about 3.0% (w/v), about 2.0% (w/v), 1.0% (w/v), about 0.95% (w/v), about 0.90% (w/v), about 0.85% (w/v), about 0.80% (w/v), about 0.75% (w/v), about 0.70% (w/v), about 0.65% (w/v), about 0.60% (w/v), or about 0.55% (w/v).

The administration of the DA-DKP containing composition may be commenced within 24 hours of diagnosis of rhinitis. The administration of the DA-DKP containing composition may be commenced at the appearance of one or more early signs of, or a predisposition to develop, rhinitis. The early signs of rhinitis include but are not limited torhinorrhea, nasal congestion, nasal itching and sneezing.

The compounds of the present invention (*i.e.,* a composition comprising DA-DKP, and preparations comprising a filtrate of DA-DKP, such as a <5000MW fraction as discussed below) may be administered to an animal patient for therapy by any suitable route of administration, including orally, nasally, parenterally (*e.g.*, intravenously, intraperitoneally, subcutaneously or intramuscularly), transdermally, intraocularly and topically (including buccally and sublingually). Preferred is oral, ocular or nasal administration for any disease or condition treatable according to the invention. Especially preferred is nasal administration.

While it is possible for a DA-DKP containing composition of the present invention to be administered alone, it is preferable to administer the DA-DKP containing composition as a pharmaceutical formulation or product. The pharmaceutical compositions of the invention comprise a compound or compounds of the invention as an active ingredient in admixture with one or more pharmaceutically-acceptable carriers and, optionally, with one or more other compounds, drugs or other materials. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the animal. Pharmaceutically-acceptable carriers are well known in the art. Regardless of the route of administration selected, the DA-DKP containing compositions of the present invention are formulated into pharmaceutically-

acceptable dosage forms by conventional methods known to those of skill in the art. See, *e.g., Remington's Pharmaceutical Sciences*.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, powders, granules or as a solution or a suspension in an aqueous or non-aqueous liquid, or an oil-in-water or water-in-oil liquid emulsions, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia), and the like, each containing a predetermined amount of a compound or compounds of the present invention as an active ingredient. Formulations of the present invention may also be administered as bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient (*i.e.,* a composition comprising DA-DKP, and preparations comprising a filtrate of DA-DKP, such as a <5000MW fraction as discussed below) is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monosterate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in

a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in microencapsulated form.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active ingredient, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Pharmaceutical formulations and products include those suitable for administration by inhalation or insufflation or for nasal administration. For administration to the upper

(nasal) or lower respiratory tract by inhalation, the DA-DKP containing compositions of the invention are conveniently delivered from a device for inhalation delivery such as an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the composition may take the form of a dry powder, for example, a powder mix of the DA-DKP containing composition of the invention and a suitable powder base, such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges, or, *e.g.*, gelatin or blister packs from which the powder may be administered with the aid of an inhalator, insufflator or a metered-dose inhaler.

For nasal administration, DA-DKP containing compositions of the invention may be administered by means of nose drops or a liquid spray, such as by means of a plastic bottle spray or atomizer or metered-dose inhaler. Liquid sprays are conveniently delivered from pressurized packs.

Nose drops may be formulated with an aqueous or nonaqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents. Drops can be delivered by means of a simple eye dropper-capped bottle or by means of a plastic bottle adapted to deliver liquid contents dropwise by means of a specially shaped closure.

Ointments, gels and creams can also be used for nasal administration of the DA-DKP containing compositions of the invention. The active ingredient may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any buffers, or propellants which may be required. The ointments, creams and gels may contain, in addition to the active ingredient, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Dosage forms for topical administration or for transdermal administration of compounds of the invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches, drops and inhalants. The active ingredient may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any buffers, or

propellants which may be required. The ointments, pastes, creams and gels may contain, in addition to the active ingredient, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof. Powders and sprays can contain, in addition to the active ingredient, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane. Transdermal patches have the added advantage of providing controlled delivery of compounds of the invention to the body. Such dosage forms can be made by dissolving, dispersing or otherwise incorporating one or more compounds of the invention in a proper medium, such as an elastomeric matrix material. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate-controlling membrane or dispersing the compound in a polymer matrix or gel. A drug-impregnated solid carrier (*e.g.,* a dressing) can also be used for topical administration.

Pharmaceutical compositions of this invention suitable for parenteral administrations comprise one or more compounds of the invention in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or non-aqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents. Also, drug-coated stents may be used.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as wetting agents, emulsifying agents and dispersing agents. It may also be desirable to include isotonic

agents, such as sugars, sodium chloride, and the like in the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monosterate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue. The injectable materials can be sterilized for example, by filtration through a bacterial-retaining filter.

The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampules and vials, and may be stored in a lyophilized condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the type described above.

While it is possible for a DA-DKP containing composition to be given alone to treat rhinitis, alternatively, the DA-DKP containing composition may be given in combination with one or more other treatments or drugs suitable for treating the rhinitis. For instance, the DA-DKP containing composition can be administered prior to, in conjunction with (including simultaneously with), or after the other treatment or drug. In the case of another drug, the drug and the DA-DKP containing composition may be administered in separate pharmaceutical compositions or as part of the same pharmaceutical composition. Suitable other drugs include antihistamines, decongestants, anti-inflammatories (steroidal and nonsteroidal), mast cell stabilizers, leukotriene modifiers and IgE blockers. Specific suitable drugs include fexofenadine, doxylamine,

diphenhydramine, triprolidine, loratidine, cetirizine, pseudophedrine, phenylephrine, aspirin, ibuprofen, naproxen, prednisone, prednisolone, and methylprednisolone. Suitable drugs for inclusion in a nasal spray are steroids (such as fluticasone propionate, mometasone, budesonite, flunisolide, triamcinolone and beclomethasone), antihistamines (such as azelastine), anticholinergics (such as ipratproium) and mast cell stabilizers (such as cromolyn).

Methods of making diketopiperazines, such as DA-DKP, are well known in the art, and these methods may be employed to synthesize the diketopiperazines of the invention. See, e.g., U.S. Patents Nos. 4,694,081, 5,817,751, 5,990,112, 5,932,579, 6,555,543; US Patent Application Publication Number 2004/0024180, PCT applications WO 96/00391 and WO 97/48685, and Smith et al., Bioorg. Med. Chem. Letters, 8, 2369-2374 (1998), the complete disclosures of which are incorporated herein by reference.

For instance, diketopiperazines, such as DA-DKP, can be prepared by first synthesizing dipeptides. The dipeptides can be synthesized by methods well known in the art using L-amino acids, D-amino acids or a combination of D- and L-amino acids. Preferred are solid-phase peptide synthetic methods. Of course, dipeptides are also available commercially from numerous sources, including DMI Synthesis Ltd., Cardiff, UK (custom synthesis), Sigma-Aldrich, St. Louis, MO (primarily custom synthesis), Phoenix Pharmaceuticals, Inc., Belmont, CA (custom synthesis), Fisher Scientific (custom synthesis) and Advanced ChemTech, Louisville, KY.

Once the dipeptide is synthesized or purchased, it is cyclized to form a diketopiperazine. This can be accomplished by a variety of techniques. For example, U.S. Patent Application Publication Number 2004/0024180 describes a method of cyclizing dipeptides. Briefly, the dipeptide is heated in an organic solvent while removing water by distillation. Preferably, the organic solvent is a low-boiling azeotrope with water, such as acetonitrile, allyl alcohol, benzene, benzyl alcohol, n-butanol, 2-butanol, t-butanol, acetic acid butylester, carbon tetrachloride, chlorobenzene chloroform, cyclohexane, 1,2-dichlorethane, diethylacetal, dimethylacetal, acetic acid ethylester, heptane, methylisobutylketone, 3-pentanol, toluene and xylene. The temperature depends on the reaction speed at which the cyclization takes place and on the type of azeotroping agent used. The reaction is preferably carried out at 50-200°C, more preferably 80-150°C. The pH range in which cyclization takes place can be easily determine by the person skilled in the art. It will advantageously be 2-9, preferably 3-7.

When one or both of the amino acids of the dipeptide has, or is derivatized to have, a carboxyl group on its side chain (e.g., aspartic acid or glutamic acid), the dipeptide is preferably cyclized as described in U.S. Patent No. 6,555,543. Briefly, the dipeptide, with the side-chain carboxyl still protected, is heated under neutral conditions. Typically, the dipeptide will be heated at from about 80°C to about 180°C, preferably at about 120°C. The solvent will be a neutral solvent. For instance, the solvent may comprise an alcohol (such as butanol, methanol, ethanol, and higher alcohols, but not phenol) and an azeotropic co-solvent (such as toluene, benzene, or xylene). Preferably, the alcohol is butan-2-ol, and the azeotropic co-solvent is toluene. The heating is continued until the reaction is complete, and such times can be determined empirically. Typically, the dipeptide will be cyclized by refluxing it for about 8-24 hours, preferably about 18 hours. Finally, the protecting group is removed from the diketopiperazine. In doing so, the use of strong acids (mineral acids, such as sulfuric or hydrochloric acids), strong bases (alkaline bases, such as potassium hydroxide or sodium hydroxide), and strong reducing agents (e.g., lithium aluminum hydride) should be avoided, in order to maintain the chirality of the final compound.

Dipeptides made on solid phase resins can be cyclized and released from the resin in one step. See, e.g., U.S. Patent No. 5,817,751. For instance, the resin having an N-alkylated dipeptide attached is suspended in toluene or toluene/ethanol in the presence of acetic acid (e.g., 1%) or triethylamine (e.g., 4%). Typically, basic cyclization conditions are preferred for their faster cyclization times.

Other methods of cyclizing dipeptides and of making diketopiperazines are known in the art and can be used in the preparation of diketopiperazines useful in the practice of the invention. See, e.g., those references listed above. In addition, many diketopiperazines suitable for use in the present invention can be made as described below from proteins and peptides. Further, diketopiperazines for use in the practice of the invention can be obtained commercially from, e.g., DMI Synthesis Ltd., Cardiff, UK (custom synthesis).

The DA-DKP composition and/or products of the present invention can be prepared from solutions containing DA-DKP, including from the commercially-available pharmaceutical compositions comprising albumin, such as human serum albumin, by well known methods, such as ultrafiltration, chromatography (size-exclusion chromatography (e.g., Centricon filtration), affinity chromatography (e.g., using a column of beads having

attached thereto an antibody or antibodies directed to the desired diketopiperazine(s) or an antibody or antibodies directed to the truncated protein or peptide), anion exchange or cation exchange), sucrose gradient centrifugation, chromatography, salt precipitation, or sonication, that will remove some or all of the albumin in the solution. The resultant DA-DKP-containing composition and/or product can be used and incorporated into pharmaceutical compositions as described above.

Using an ultrafilration separation method, a human serum albumin composition can be passed over an ultrafiltration membrane having a molecular weight cut-off that retains the albumin while the DA-DKP passes into the resulting filtrate or fraction. This filtrate may comprise components having molecular weights less than about 50 kDA, less than about 40 kDa, less than 30 kDa, less than about 20 kDa, less than about 10 kDa, less than about 5 kDa, less than about 3 kDa. Preferably, the filtrate comprises components having molecular weights less than about 5 kDa (also referred to as "<5000MW"). This <5000MW fraction or filtrate contains DA-DKP which is formed after the dipeptide aspartate-alanine is cleaved from albumin and subsequently cyclized into the diketopiperazine. The <5000MW Fraction can be prepared as described in U.S. Patent No. 7,732,403, the complete disclosure of which is incorporated herein by reference.

Physiologically-acceptable salts of the DA-DKP of the invention may also be used in the practice of the invention. Physiologically-acceptable salts include conventional non-toxic salts, such as salts derived from inorganic acids (such as hydrochloric, hydrobromic, sulfuric, phosphoric, nitric, and the like), organic acids (such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, glutamic, aspartic, benzoic, salicylic, oxalic, ascorbic acid, and the like) or bases (such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation or organic cations derived from N,N-dibenzylethylenediamine, D-glucosamine, or ethylenediamine). The salts are prepared in a conventional manner, e.g., by neutralizing the free base form of the compound with an acid.

Kits comprising the pharmaceutical products of the present invention are also provided. The kits can comprise a DA-DKP composition formulated for nasal administration. The DA-DKP can be prepared as described herein, such as by removing albumin from a solution of a human albumin composition. The kits may contain unit-dose or multi-dose sealed containers, for example, squeeze bottles, syringes, a dropper, a spray device, an atomizer device, an aerosolize, a nebulizer, an insufflators or pressurized

packs. The kits may be stored in a condition, wherein the contents are ready for direct use. Alternately, the kits may be stored in a lyophilized condition requiring only the addition of the sterile liquid carrier, for example water, immediately prior to use.

As used herein, "a" or "an" means one or more.

As used herein, "comprises" and "comprising" include within their scope all narrower terms, such as "consisting essentially of" and "consisting of" as alternative embodiments of the present invention characterized herein by "comprises" or "comprising". In regard to use of "consisting essentially of", this phrase limits the scope of a claim to the specified steps and materials and those that do not materially affect the basic and novel characteristics of the invention disclosed herein.

Additional objects, advantages and novel features of the present invention will become apparent to those skilled in the art by consideration of the following non-limiting examples. The following experimental results are provided for purposes of illustration and are not intended to limit the scope of the invention.

EXAMPLE

A Phase Ib randomized, double-blinded, placebo-controlled, parallel group study was performed to evaluate the efficacy of the <5000MW Fraction for treating allergic rhinitis in adult humans. Briefly, the study was performed as follows.

The subjects were male or female humans, 18-65 years of age, with a history of allergic rhinitis that had been receiving therapy (continuous or intermittent) for more than 1 year. Each subject had a demonstrated sensitivity to at least one seasonal allergen (tree/grass pollen) known to induce allergic rhinitis through a standard skin test. Each subject had a minimum subject-reported reflective Total Nasal Symptom Score (rTNSS) of $\geq$ 5 on the day of the Screening Visit (day 1).

During a seven-day wash out period (days 1-7), the subjects received no treatment of any type and recorded their symptoms twice a day (in the morning before bathing, consumption of food or beverages or strenuous activities and 12 hours later) using the following scale:

- 0 = absent (no sign/symptom present)
- 1 = mild (sign/symptom clearly present, but minimal awareness; easily tolerated)
- 2 = moderate (definite awareness of sign/symptom that is bothersome but tolerable)

• 3 = severe (sign/symptom that is hard to tolerate; causes interference with activities of daily living and/or sleeping)

The subjects were randomized into treatment groups (<5000MW Fraction or placebo (i.e. saline)) after the wash out period (on day 8).

Treatment was begun on day 8 and continued through day 17 (10 days total). During treatment, the subjects again recorded their symptoms twice daily (as described above and before administration of the test medications in the morning) using the above scale. The subjects administered 0.3 ml of one of the test medications, 0.15 ml per nostril, once daily in the morning, immediately after recording their symptoms. The first administration of test medications was supervised by test site personnel. The test medications were the <5000MW Fraction (containing 3.72% DA-DKP) used as a nasal spray and placebo spray (containing normal saline, 0.9% NaCl) in identical intranasal spray format.

Efficacy of the test medications was assessed as follows:

The measures of effectiveness in this study included the subject-reported Total Nasal Symptom Score (TNSS). The TNSS is defined as the sum of the subject-reported symptom scores for the four nasal symptoms: rhinorrhea (runny nose), nasal congestion, nasal itching, and sneezing. Each score is assessed on a severity scale ranging from 0 to 3 as defined above.

The subjects were asked to assess both reflective TNSS (i.e., an evaluation of symptom severity over the past 12 hours prior to the recording of the score) and instantaneous TNSS (i.e., an evaluation of the symptom severity over the last 10 minutes).

The reflective and instantaneous TNSS are defined as the sum of the subject-reported symptom scores for the four nasal symptoms. Each subject recorded the symptom scores in the subject's diary. For each score, information recorded in the diary included the following:

- rhinorrhea (runny nose)
- nasal congestion
- nasal itching
- sneezing

The severity scale for each symptom evaluation is given above.

Mean reflective (r) and instantaneous (i) subject-reported total nasal symptom scores (TNSS) were calculated for each subject. The mean TNSS is the average of all AM

and PM daily scores (each score is ranked on a scale from 0-12) during the baseline (baseline efficacy value) and the treatment period (double-blind efficacy value).

The change in efficacy was calculated as the change in the double-blind value – baseline value as follows:

- Change in rTNSS: mean double-blind rTNSS – mean baseline rTNSS
- Change in iTNSS: mean double-blind iTNSS – mean baseline iTNSS

Student's t-test: mean (SD) difference between treatment groups for the following:

- Mean change in (rTNSS/iTNSS)
- Mean change in AM (rTNSS/iTNSS)
- Mean change in PM (rTNSS/iTNSS)
- Mean change in individual symptom scores for each of the four nasal symptoms.

The results obtained by analysis using the Student's t-test are provided in Tables 1-5 and are summarized below (number of subjects who completed the trial at the time of the interim analysis: n=20; placebo, n=10 (Intent to Treat (ITT) population; >5000MW Fraction, n=10 (ITT population).

rTNSS: <5000MW Fraction showed a 2.4 point decrease in reflective symptom severity compared to 1.7 point decrease with placebo (Table 2). There was 35% improvement in rTNSS with <5000MW Fraction versus 23% improvement with placebo. The largest improvements were with sneezing and runny nose symptoms (Table 3). 6/7 efficacy measures showed a larger improvement with <5000MW Fraction than placebo (all assessments but itchy nose symptom). There was significant rTNSS improvement in subset with baseline rTNSS < median (7.5).

Table 1 Baseline efficacy variables, mean (SD)

Efficacy Variable	Placebo	<5000MW Fraction	T-test p value
rTNSS	7.46 (1.71)	6.84 (1.39)	0.39
iTNSS	7.40 (1.71)	6.45 (1.11)	0.16
PNSS (screening)	6.50 (1.43)	5.60 (1.65)	0.21
PNSS (randomization)	6.90 (1.60)	5.50 (0.85)	**0.03**

PNSS = Physician Nasal Symptoms Score; TNSS = Total Nasal Symptoms Score

Table 2 Mean change in rTNSS

Efficacy Variable	Saline n=10 Mean (SD)	<5000MW Fraction n=10 (SD)	T-test p value
rTNSS	-1.71 (1.82)	-2.37 (2.06)	0.46
AM rTNSS	-1.68 (1.69)	-2.25 (2.00)	0.51
PM rTNSS	-1.72 (1.95)	-2.03 (2.08)	0.74

Table 3 Mean Change in Reflective Symptoms

Symptom	Saline n=10 Mean (SD)	<5000MW Fraction n=10 (SD)	T-test p value
rRunny nose	-0.33 (0.8)	-0.60 (0.8)	0.45
rStuffy nose	-0.58 (0.6)	-0.59 (0.5)	0.97
rItchy nose	-0.54 (0.6)	-0.53 (0.6)	0.97
rSneezing	-0.26 (0.3)	-0.65 (0.7)	0.13

iTNSS: <5000MW Fraction showed a 2.0 point decrease in instantaneous symptom severity compared to 1.7 point decrease with placebo (Table 4). 5/7 efficacy measures showed a larger improvement with <5000MW Fraction than with placebo. The largest improvements were with sneezing nose symptoms (Table 5). Smaller improvements were seen with instantaneous versus reflective symptoms.

Table 4 Mean Change in iTNSS

Efficacy variable	**Saline n=10 Mean (SD)**	**<5000MW Fraction n=10 (SD)**	**T-test p value**
iTNSS	-1.68 (1.84)	-1.97 (2.06)	0.75
AM iTNSS	-1.55 (1.69)	-1.84 (1.94)	0.73
PM iTNSS	-1.50 (1.61)	-1.56 (1.98)	0.94

Table 5 Mean Change in Instantaneous Symptoms

Symptom	**Placebo Mean (SD)**	**<5000MW Fraction (SD)**	**T-test p value**
iRunny nose	-0.27 (0.8)	-0.37 (0.8)	0.78
iStuffy nose	-0.54 (0.4)	-0.53 (0.6)	0.98
iItchy nose	-0.63 (0.6)	-0.46 (0.6)	0.53
iSneezing	-0.24 (0.6)	-0.61 (0.5)	0.16

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following exemplary claims.

WHAT IS CLAIMED:

1. A method of treating rhinitis comprising administering an effective amount of a pharmaceutical composition comprising a diketopiperazine with amino acid side chains of aspartic acid and alanine (DA-DKP), to an animal in need thereof.
2. The method of Claim 1, wherein the rhinitis is allergic rhinitis.
3. The method of Claim 1, wherein the effective amount of the DA-DKP in the composition is from about 100 μg to about 3000 μg per day.
4. The method of Claim 1, wherein the effective amount of the DA-DKP in the composition is from about from about 500 μg to about 1500 μg per day.
5. The method of Claim 1, wherein administration of the composition comprising DA-DKP, is commenced within 24 hours of diagnosis of rhinitis.
6. The method of Claim 1, wherein administration of the composition comprising DA-DKP, is commenced at the appearance of one or more early signs of, or a predisposition to develop, rhinitis.
7. The method of Claim 6, wherein the one or more early signs of rhinitis are selected from the group consisting of rhinorrhea, nasal congestion, nasal itching and sneezing.
8. The method of Claim 1, wherein the DA-DKP is in a composition prepared by removing albumin from a solution of a human serum albumin composition.
9. The method of Claim 8, wherein the step of removing the albumin comprises treating the human serum albumin composition by a separation method selected from the group consisting of ultrafiltration, sucrose gradient centrifugation, chromatography, salt precipitation, and sonication.
10. The method of Claim 9, wherein the step of removing comprises passing the human serum albumin composition over an ultarfiltration membrane with a molecular weight cut off that retains the albumin, and wherein the resulting filtrate comprises DA-DKP.
11. The method of Claim 10, wherein the ultrafiltration membrane has molecular weight cutoff of less than 50 kDa, less than 40 kDa, less than 30 kDA, less than 20 kDa, less than 10 kDa, less than 5 kDa or less than 3 kDA.
12. The method of Claim 1, wherein the composition comprising DA-DKP is administered in combination with a second drug suitable for treating rhinitis.

13. The method of Claim 12, wherein the second drug suitable for treating rhinitis is selected from the group consisting of antihistamines, decongestants, anti-inflammatories, mast cell stabilizers, leukotriene modifiers and IgE blockers.
14. A pharmaceutical product, comprising a DA-DKP containing composition formulated for administration by a route selected from the group consisting of inhalation, insufflation and nasal administration to the nose.
15. The pharmaceutical product of Claim 14, wherein the DA-DKP containing composition is formulated for administration by inhalation is packaged in a device selected from the group consisting of insufflators, nebulizers, pressurized packs, squeeze bottle, a syringe, a dropper, a spray device, an atomizer device, and an aerosolizer.
16. The pharmaceutical product of Claim 15, wherein the pressurized pack comprises a propellant selected from the group consisting of dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, and carbon dioxide.
17. The pharmaceutical product of Claim 14, wherein the DA-DKP containing composition formulated for administration by inhalation or insufflation comprises a powder mix of the DA-DKP containing composition and a powder base.
18. The pharmaceutical product of Claim 17, wherein the powder mix is in a dosage form selected from the group consisting of capsules, cartridges, gelatin packs and blister packs.
19. The pharmaceutical product of Claim 18, wherein the powder mix is delivered by a device selected from the group consisting of an inhalator, insufflator and metered-dose inhaler.
20. The pharmaceutical product of Claim 14, wherein the DA-DKP containing composition formulated for nasal administration is in a form of drops or sprays.
21. The pharmaceutical product of Claim 20, wherein the DA-DKP comprises from about 0.1% (w/v) to about 10% (w/v) of the composition.
22. The pharmaceutical product of Claim 20, wherein the drops or sprays are contained within an intranasal delivery system.
23. The pharmaceutical product of Claim 22, wherein the intranasal delivery system comprises an atomizing device.

24. The pharmaceutical product of Claim 23, wherein the atomizing device comprises a bottle and a pump.
25. The pharmaceutical product of Claim 24, wherein the pump is a metered dose pump.
26. The pharmaceutical product of Claim 25, wherein the metered dose pump delivers an intranasal volume of the DA-DKP containing composition of about 0.15 ml per pump.
27. The pharmaceutical product of Claim 20, further comprising an aqueous or non-aqueous base comprising one or more agents selected from the group consisting of dispersing agents, solubilizing agents, and suspending agents.
28. The pharmaceutical product of Claim 14, wherein the DA-DKP containing composition formulated for nasal administration is in a form selected from the group consisting of ointments, gels and creams.
29. The pharmaceutical product of Claim 28, wherein the DA-DKP containing composition further comprises excipients selected from the group consisting of animal fats, vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycol, silicones, bentonites, silicic acid, talc, zinc oxide and mixtures thereof.
30. The pharmaceutical product of Claim 14, further comprising an absorption or permeation enhancer.
31. The pharmaceutical product of Claim 14, further comprising a thickening agent or viscosity enhancer to increase the residence time of the DA-DKP containing composition in the nose.
32. The pharmaceutical product of Claim 14, further comprising a pharmaceutically-acceptable carrier.
33. The pharmaceutical product of Claim 14, wherein the DA-DKP is prepared by removing albumin from a solution of a human serum albumin composition.
34. The pharmaceutical product of Claim 33, wherein the step of removing the albumin comprises treating the human serum albumin composition by a separation method selected from the group consisting of ultrafiltration, sucrose gradient centrifugation, chromatography, salt precipitation, and sonication.
35. The pharmaceutical product of Claim 34, wherein the step of removing comprises passing the human serum albumin composition over an

ultarfiltration membrane with a molecular weight cut off that retains the albumin, and wherein the resulting filtrate comprises DA-DKP.

36. The pharmaceutical product of Claim 35, wherein the ultrafiltration membrane has molecular weight cutoff of less than 50 kDa, less than 40 kDa, less than 30 kDA, less than 20 kDa, less than 10 kDa, less than 5 kDa or less than 3 kDA.