AMMONIA OXIDIZING MICROORGANISMS FOR USE AND DELIVERY TO THE INTRANASAL SYSTEM

Cross-Reference to Related Applications

This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application Serial No. 62/397,642 as filed on September 21, 2016, the entire disclosure of which is hereby incorporated herein by reference in its entirety for all purposes.

Field of the Technology

Aspects relates generally to the microbiome and, more specifically, to the restoration of ammonia oxidizing microorganisms in relation to the microbiome.

Background

Bacteria and other microorganisms are ubiquitous in the environment. The discovery of pathogenic bacteria and the germ theory of disease have had a tremendous effect on health and disease states. Microorganisms are a normal part of the environment of all living things and may be beneficial. In the nasal passages, inhaled air passes over specialized nasal structures. Microorganisms, ambient molecules, and particles elicit responses in the nasal passages and become trapped in a layer of high viscosity mucus. The internal nasal surface is characterized by groups of ciliated cells which act to transport the layer of high viscosity mucus for local or systemic delivery.

Summary

In one aspect, there is provided a use of a preparation comprising AOM in the manufacture of a medicament for treating neurogenic inflammation (e.g., oxidative stress), a neurological disorder, a nasal or sinus disorder, or a systemic inflammatory condition in a subject, the use comprising:

an effective amount of the preparation comprising AOM to be administered to the nasal cavity of the subject,

wherein the AOM colonize a target tissue of the nasal cavity of the subject.

In accordance with one or more examples, a method of introducing ammonia oxidizing microorganisms (AOM) to a subject is disclosed. The method may comprise intranasally administering a preparation comprising AOM to the subject.

In some examples, intranasal administration may comprise topical application, inhalation, instillation, or olfactory transfer administration.

In accordance with one or more examples, a method of delivering AOM to a nasal cavity of a subject is disclosed. The method may comprise administering an effective amount of a

preparation comprising AOM to the nasal cavity of the subject, wherein the AOM colonize a target tissue of the nasal cavity.

In accordance with one or more examples, a method of treating a neurogenic inflammation in a subject is disclosed. The neurogenic inflammation may comprise, for example, oxidative stress. The method may comprise administering to the subject an effective amount of a preparation comprising AOM, thereby treating the neurogenic inflammation in the subject.

In accordance with one or more examples, a method of treating a neurological disorder in a subject is disclosed. The method may comprise administering to the subject an effective amount of a preparation comprising AOM, thereby treating the neurological disorder in the subject.

In accordance with one or more examples, a method of treating a nasal or sinus disorder in a subject is disclosed. The method may comprise administering to the subject an effective amount of a preparation comprising AOM, thereby treating the nasal or sinus disorder in the subject.

In accordance with one or more examples, a method of treating a systemic inflammatory condition in a subject is disclosed. The method may comprise intranasally administering to the subject an effective amount of a preparation comprising AOM, thereby treating the systemic inflammatory condition in the subject. In some examples, the systemic inflammatory condition is associated with one or more of: headaches (e.g., migraines), cardiovascular diseases (e.g., hypertension), inflammation, immune responses, autoimmune disorders, liver diseases, infections, neurological diseases, psychiatric disorders, nitric oxide disorders, urea cycle disorders, congestion, vasodilation disorders, skin diseases, wound healing, reactions to insect bites, ophthalmic disorders, connective tissue disorders, and certain viral, bacterial, or fungal infections.

In accordance with one or more examples, a method of treating a headache or a migraine headache in a subject is disclosed. The method may comprise intranasally administering to the subject an effective amount of a preparation comprising AOM, thereby treating the headache or migraine headache in the subject.

In some examples, an amount and/or a frequency of administration is sufficient to increase mucus thickness in at least a portion of the nasal cavity of the subject. Administering the preparation may result in anti-triggering of ischemic pre-conditioning or modulation of an ATP

level in the subject. An amount and/or a frequency of administration may be sufficient to induce ischemic anti-triggering in the subject.

In at least some examples, the preparation comprising AOM is administered intranasally to a nasal cavity of a subject. The subject may have a substantially cleared nasal cavity when the preparation is administered. The preparation may be administered subsequent to administration of an antibiotic or a nasal cavity cleansing preparation. In some embodiments, a target percentage of administered AOM are transferred to a central nervous system (CNS) of the subject. The method may further comprise administering water or a buffer solution, e.g., an aqueous buffer solution to the subject subsequent to administering the preparation.

In some examples, administering the preparation results in decongestion, decreased sinus pressure, or modulation of an inflammatory response. In at least some examples, the neurological disorder is an inflammatory condition. The inflammatory condition may be a nasal or sinus disorder. The nasal or sinus disorder may be allergic rhinitis.

The inflammatory condition may be neurogenic inflammation, e.g., associated with headache, neuropathy (e.g., diabetic neuropathy, peripheral neuropathy, Lewy body neuropathy), epilepsy, systemic sclerosis, multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's Disease, Parkinson's Disease, white matter hyperintensities, diabetic retinopathy, anxiety, post-traumatic stress disorder, chronic fatigue syndrome, fibromyalgia, depression, insomnia, arthritis, rheumatoid arthritis, allergic rhinitis, dilative cardiomyopathy, atherosclerosis, cardioprotection, heart failure, hypertension (e.g., pulmonary hypertension, gestational hypertension, portal hypertension), eclampsia, pre-eclampsia, capillary rarefaction, peripheral vasculopathy, gestational diabetes, type 2 diabetes, obesity, metabolic syndrome, kidney failure, liver failure, pancreatitis, or hepatitis.

In some examples, the nasal or sinus disorder may relate to an allergen, a bacterial infection, or a viral infection, e.g., the coronavirus, rhinovirus, meningitis, or influenza. The inflammatory condition may be associated with an injury, cancer, or an infection.

In some examples, administration may precede or follow a medical procedure, e.g., a catheterization, endoscopy, intubation, e.g., nasogastric intubation, administration of a nasal cannula, or a dental procedure. The inflammatory condition may be associated with or follow an injury, e.g., spinal cord injury, head trauma, or brain injury.

In some examples, the nasal or sinus disorder may be characterized by congestion or sinus pressure. In some examples, the neurological disorder may be a degenerative disorder or a genetic disorder. The neurological disorder may comprise a psychological disorder.

In some examples, administration may be to a deposit tissue or directly to a target tissue. A deposit tissue, target tissue, or both may be a mucous membrane of the subject. The deposit tissue, target tissue, or both may be associated with a nasal cavity of the subject. The deposit tissue, target tissue, or both may be a nasal cavity, septal wall, nasal valve, nostril, nasopharanyx, vestibular area, turbinate (e.g., inferior, middle, superior), meatus (e.g., inferior, middle, superior), concha (e.g., inferior, middle, superior), maxillary sinus, sphenoidal sinus, sphenoethmoidal recess, ethmoidal bulla, semi-lunar hiatus, nasolacrimal duct, frontonasal duct, or olfactory region of the subject.

In some examples, the target tissue may be associated with a desired local effect. The target tissue may be associated with a desired systemic effect. For example, a desired systemic effect may involve treatment of one or more of: headaches, cardiovascular diseases, inflammation, immune responses, autoimmune disorders, liver diseases, infections, neurological diseases, psychiatric disorders, nitric oxide disorders, urea cycle disorders, congestion, vasodilation disorders, skin diseases, wound healing, reactions to insect bites, ophthalmic disorders, connective tissue disorders, and certain viral, bacterial, or fungal infections.

In some examples, administering an effective amount of the preparation may promote endothelial function. Administering an effective amount of the preparation may change or alter a level of nitrite or NO at a target tissue or in circulation. Administering an effective amount of the preparation may modulate a microbiome associated with the intranasal system of the subject. Administering an effective amount of the preparation may modulate a microbiome associated with the CNS of the subject. Administering an effective amount of the preparation may modulate a systemic microbiome associated with a remote system, e.g., gastrointestinal system, circulatory system, respiratory system, endocrine system, or immune system, of the subject.

In some examples, administration may be device-assisted. The preparation may be administered prior to onset, during incidence, or subsequent to the subsiding of a gastrointestinal condition. The preparation may be administered in response to an inflammatory symptom, trigger or warning sign, e.g. discomfort, a change in sinus pressure, or a stress state. A method

may involve determining if the subject is in need of treatment for a neurological disorder. A method may involve determining if the subject is in need of treatment for a nasal or sinus disorder.

In some examples, the preparation may be administered as a solution, suspension, emulsion, ointment, bougie, powder, gel, hydrogel, or liquid, e.g. drop, spray, aerosol, or mist. The preparation may be formulated as a drop, spray, aerosol, or mist. The preparation may include microspheres or microcapsules. The preparation may be formulated to be compatible with the mucous membrane of the nasal cavity of the subject. The preparation may be formulated for immediate release or extended release. The preparation may be formulated to deliver nitrite or NO to a target tissue, locally or systemically. The preparation may be formulated for transmucosal delivery and/or circulation, *e.g.* locally or systemically.

In some examples, administration may reduce inflammation, congestion, sinusitis, asthma, sneezing, sinus pressure, or discomfort in the subject.

In some examples, a treatment method may further comprise administering a second amount of the preparation to the subject. In at least some examples, the preparation may be administered as part of a combination therapy. The method may further comprise administering a second treatment in combination with the preparation. The preparation may be administered for a period of time prior to initiating the second treatment, concurrently with the second treatment, or for a period of time subsequent to ceasing the second treatment. The second treatment may be administered via an alternate mode of administration, e.g. via inhalation or enteral technique. The subject may have a therapeutic level of a second treatment. The preparation may be administered in conjunction with an anti-inflammatory agent. The preparation may be administered in conjunction with a medical approach that treats, e.g., is approved to treat or is commonly used to treat, the relevant disease or disorder, or a symptom of the relevant disease or disorder. The preparation may be administered before or after a surgical or diagnostic procedure. The second treatment may comprise a surgical procedure. The preparation may be administered in conjunction with decongestant, probiotic, therapeutic, exercise, or stress management.

In at least some examples, the preparation may be administered in combination with a therapeutic treatment for headache, neuropathy (e.g., diabetic neuropathy, peripheral neuropathy,

Lewy body neuropathy), epilepsy, systemic sclerosis, multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's Disease, Parkinson's Disease, white matter hyperintensities, diabetic retinopathy, anxiety, post-traumatic stress disorder, chronic fatigue syndrome, fibromyalgia, depression, insomnia, arthritis, rheumatoid arthritis, allergic rhinitis, dilative cardiomyopathy, atherosclerosis, cardioprotection, heart failure, hypertension (e.g., pulmonary hypertension, gestational hypertension, portal hypertension), eclampsia, pre-eclampsia, capillary rarefaction, peripheral vasculopathy, gestational diabetes, type 2 diabetes, obesity, metabolic syndrome, kidney failure, liver failure, pancreatitis, or hepatitis. The preparation may be administered in conjunction with nitrite, nitrate, and/or NO.

In some examples, the effective amount is a therapeutically effective dose of AOM. The therapeutically effective dose of AOM is about or greater than about 1 x 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, or 10^{14} CFU. The preparation may be administered as an analgesic and/or as a prophylactic. The preparation may be self-administered. The preparation may be administered about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 times per day. The preparation may be administered for about 1-3, 3-5, 5-7, 7-9, 5-10, 10-14, 12-18, 12-21, 21-28, 28-35, 35-42, 42-49, 49-56, 46-63, 63-70, 70-77, 77-84, or 84-91 days. The preparation may be administered within 30, 60, 90, 120, 150, or 180 minutes of the subject waking from sleep. The preparation may be administered within 30, 60, 90, 120, 150, or 180 minutes prior to the subject sleeping. The preparation may be administered within 30, 60, 90, 120, 150, or 180 minutes of the subject eating. The preparation may be administered 30, 60, 90, 120, 150, or 180 minutes before the subject cleanses or showers.

In some examples, the subject may be female. In other examples, the subject may be male. The subject may be characterized as one of the following ethnicity/race: Asian, black or African American, Hispanic or Latino, white, or multi-racial. The subject may have a disrupted microbiome. The subject may be of an age less than 1, or between 1-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, or over 60 years.

In some examples, the preparation may comprise AOM in a buffer solution, e.g., an aqueous buffer solution. The buffer solution, e.g., aqueous buffer solution, comprises disodium phosphate and magnesium chloride, for example, 50 mM Na_2HPO_4 and 2 mM $MgCl_2$ in water. The buffer solution e.g., aqueous buffer solution, consisting essentially of disodium phosphate

and magnesium chloride, for example, 50 mM Na_2HPO_4 and 2 mM $MgCl_2$ in water. The buffer solution, e.g., aqueous buffer solution, consists of disodium phosphate and magnesium chloride, for example, 50 mM Na_2HPO_4 and 2 mM $MgCl_2$ in water. The preparation may be characterized by a physiological pH level. The preparation may further comprise or be administered concurrently with a compound that promotes growth or metabolism of the AOM, NO production, and/or urease activity. In at least some examples, the preparation may comprise at least one of ammonia, ammonium salts, and urea. The preparation may comprise a controlled release material, e.g., slow release material. The preparation may further comprise an excipient, e.g., a pharmaceutically acceptable excipient. The excipient may comprise an absorption or penetration enhancer, preservative, antioxidant, buffer, chelating agent, ion exchange agent, solubilizing agent, suspending agent, thickener, surfactant, wetting agent, tonicity-adjusting agent, enzyme inhibitor, or vehicle for proper drug delivery. In at least some aspects, the preparation may comprise a mucoadhesive agent, disintegrant, chelator, coating agent, modified-release product, or filler. The preparation may be substantially free of other organisms.

In some examples, the preparation may comprise between about 1 x 10^3 CFU/mL to about 1 x 10^{14} CFU/mL AOM. The preparation may comprise between about 1 x 10^9 CFU/mL to about 10 x 10^9 CFU/mL AOM. The AOM may comprise ammonia oxidizing bacteria (AOB). In some aspects, the AOM may consist essentially of AOB. In at least some aspects, the AOM may consist of AOB. The AOM may comprise *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosocystis*, *Nitrosolobus*, *Nitrosovibrio*, and combinations thereof. The AOM may be *Nitrosomonas eutropha* (*N. eutropha*). The AOM may be *N. eutropha* D23, having ATCC accession number PTA-121157. In at least some examples, the AOM may comprise ammonia oxidizing archaea (AOA). The AOM may be capable of converting ammonia or ammonium to nitrite at a rate of at least about 1 pmol/min/mg protein. The AOM may be capable of converting ammonia or ammonium to nitrite at a rate of at least about 0.1 nmol/min/mg protein.

In some examples, the preparation may be administered, e.g., intranasally to a first tissue, e.g. a deposit tissue. The first tissue may be the target tissue. In at least some examples, the first tissue is other than the target tissue, e.g., the preparation may be applied to a first tissue and the preparation, or a product of the preparation, e.g., NO, may be transported, e.g., by diffusion, to a second tissue, e.g. the target tissue.

In some examples, the treatment may comprise a surgical procedure. The excipient may comprise an anti-adherent, binder, coat, disintegrant, filler, flavor, color, lubricant, glidant, sorbent preservative, or sweetener.

In some examples, a biome-friendly product may be used in connection with the administered preparation comprising AOM.

In accordance with one or more examples, a preparation comprising AOM, as recited in any of the preceding claims, may be for intranasal administration to a subject.

In some examples, the preparation may be a nasal drop, spray, aerosol, or mist.

In accordance with one or more examples, a preparation may comprise AOM. The preparation may be for treatment of a neurological disorder in a subject. The preparation may be for treatment of a nasal or sinus disorder in a subject.

In some examples, the preparation may be packaged for single use. In some examples, the preparation may be packaged for multiple use.

In some examples, the preparation may further comprise other organisms, e.g., a community of organisms.

In accordance with one or more examples, a device may be configured to administer a preparation comprising AOM to a target tissue of a nasal cavity of a subject.

In accordance with one or more examples, a kit may comprise a preparation comprising AOM, e.g., for delivery to an intranasal system of a subject or for treatment of a neurological disorder or nasal or sinus disorder in a subject.The disclosure contemplates all combinations of any one or more of the foregoing examples, as well as combinations with any one or more of the examples set forth in the detailed description and any examples.

Detailed Description

In accordance with one or more embodiments, the present disclosure provides for various methods or modes of introducing ammonia oxidizing microorganisms to a subject. These methods or modes comprise administering to a subject ammonia oxidizing microorganisms, for example, a preparation, composition, formulation, or product comprising ammonia oxidizing microorganisms. In at least some embodiments, ammonia oxidizing microorganisms may

CLAIMS

1. Use of a preparation comprising AOM in the manufacture of a medicament for treating neurogenic inflammation (e.g., oxidative stress), a neurological disorder, a nasal or sinus disorder, or a systemic inflammatory condition in a subject, the use comprising:

an effective amount of the preparation comprising AOM to be administered to the nasal cavity of the subject,

wherein the AOM colonize a target tissue of the nasal cavity of the subject.

2. The use according to claim 1, wherein the neurological disorder:

(i) is an inflammatory condition, optionally a nasal or sinus disorder;

(ii) is a degenerative disorder or a genetic disorder; or

(iii) comprises a psychological disorder.

3. The use according to claim 1, wherein the nasal or sinus disorder:

(i) is allergic rhinitis;

(ii) relates to an allergen, a bacterial infection, or a viral infection, e.g., the coronavirus, rhinovirus, meningitis, or influenza; or

(iii) is characterized by congestion or sinus pressure.

4. The use according to claim 1, wherein the systemic inflammatory condition is associated with one or more of: headaches (e.g., migraines), cardiovascular diseases (e.g., hypertension), inflammation, immune responses, autoimmune disorders, liver diseases, infections, neurological diseases, psychiatric disorders, nitric oxide disorders, urea cycle disorders, congestion, vasodilation disorders, skin diseases, wound healing, reactions to insect bites, ophthalmic disorders, connective tissue disorders, and certain viral, bacterial, or fungal infections.

5. The use according to claim 1, wherein the inflammatory condition:

(i) is neurogenic inflammation, e.g., associated with headache, neuropathy (e.g., diabetic neuropathy, peripheral neuropathy, Lewy body neuropathy), epilepsy, systemic sclerosis, multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's Disease, Parkinson's Disease, white matter hyperintensities, diabetic retinopathy, anxiety, post-traumatic stress disorder, chronic fatigue syndrome, fibromyalgia, depression, insomnia, arthritis, rheumatoid arthritis, allergic rhinitis, dilative cardiomyopathy, atherosclerosis, cardioprotection, heart failure, hypertension (e.g., pulmonary hypertension, gestational hypertension, portal hypertension), eclampsia, pre-eclampsia, capillary rarefaction, peripheral vasculopathy, gestational diabetes, type 2 diabetes, obesity, metabolic syndrome, kidney failure, liver failure, pancreatitis, or hepatitis;
(ii) is associated with an injury, cancer, or an infection; or
(iii) follows an injury, e.g., spinal cord injury, head trauma, or brain injury.

6. The use according to claim 1, wherein the preparation comprising AOM is to be administered intranasally to a nasal cavity of a subject, optionally wherein intranasal administration comprises topical application, inhalation, instillation, or olfactory transfer administration.

7. The use according to claim 1, wherein:
(i) an amount and/or a frequency of administration is sufficient to increase mucus thickness in at least a portion of the nasal cavity of the subject;
(ii) the preparation is to be administered to substantially clear the nasal cavity of the subject;
(iii) the preparation is to be administered subsequent to an antibiotic or a nasal cavity cleansing preparation to be administered;
(iv) a target percentage of administered AOM are transferred to a central nervous system (CNS) of the subject; or
(v) the preparation to be administered will result in decongestion, decreased sinus pressure, or modulation of an inflammatory response.

8. The use according to claim 1, wherein:

(i) an amount and/or a frequency of administration is sufficient to induce ischemic anti-triggering in the subject;

(ii) administration reduces inflammation, congestion, sinusitis, asthma, sneezing, sinus pressure, or discomfort in the subject;

(iii) the use further comprises a second amount of the preparation to be administered to the subject;

(iv) the therapeutically effective dose of AOM is about or greater than about 1 x 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, or 10^{14} CFU;

(v) the preparation comprises AOM in a buffer solution, e.g., an aqueous buffer solution, optionally comprising disodium phosphate and magnesium chloride, for example, 50 mM Na_2HPO_4 and 2 mM $MgCl_2$ in water; or

(vi) the preparation is to be administered to a first tissue, e.g. a deposit tissue, and wherein the first tissue is other than the target tissue, e.g., the preparation is to be applied to a first tissue and the preparation, or a product of the preparation, e.g., NO, is transported, e.g., by diffusion, to a second tissue, e.g. the target tissue; or

(vii) a biome-friendly product is used in connection with the preparation comprising AOM.

9. The use according to claim 1, wherein an effective amount of the preparation to be administered:

(i) promotes endothelial function;

(ii) changes or alters a level of nitrite or NO at a target tissue or in circulation;

(iii) modulates a microbiome associated with the intranasal system of the subject;

(iv) modulates a microbiome associated with the CNS of the subject; or

(v) modulates a systemic microbiome associated with a remote system, e.g., gastrointestinal system, circulatory system, respiratory system, endocrine system, or immune system, of the subject.

10. The use according to claim 1, wherein the preparation:

(i) is formulated to be compatible with the mucous membrane of the nasal cavity of the subject;

(ii) is formulated for immediate release or extended release;

(iii) is to be administered as part of a combination therapy; or

(iv) is substantially free of other organisms; or

(v) comprises between about 1 x 10^3 CFU/mL to about 1 x 10^{14} CFU/mL AOM.

11. The use according to claim 1, wherein the AOM:

(i) comprise ammonia oxidizing bacteria (AOB);

(ii) comprise *Nitrosomonas*, *Nitrosococcus*, *Nitrosospira*, *Nitrosocystis*, *Nitrosolobus*, *Nitrosovibrio*, and combinations thereof;

(iii) is *Nitrosomonas eutropha* (*N. eutropha*);

(iv) is *N. eutropha* D23, having ATCC accession number PTA-121157; or

(v) are capable of converting ammonia or ammonium to nitrite at a rate of at least about 1 pmol/min/mg protein, e.g., at least about 0.1 nmol/min/mg protein.

12. The use according to claim 1:

(i) wherein the preparation is a nasal drop, spray, aerosol, or mist;

(ii) wherein the preparation is formulated for use in the treatment of a neurological disorder in a subject;

(iii) wherein the preparation is formulated for use in the treatment of a nasal or sinus disorder in a subject; or

(iv) wherein the preparation comprises AOM and other organisms, e.g., a community of organisms.