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(54) **Title**: METHOD FOR TREATING RHINITIS AND SINUSITIS BY RHAMNOLIPIDS

(57) **Abstract**: The present invention is directed to a method for treating rhinitis, or sinusitis, in a subject. The methods comprise the steps of: identifying a subject in need thereof, and administering intranasally to the subject a formulation comprising an effective amount of rhamnolipid, whereby the symptoms in the subject are reduced. The formulation preferably comprises rhamnolipid 1, rhamnolipid 2, or the combination. The present invention is particularly useful for treating allergic rhinitis.

METHOD FOR TREATING RHINITIS AND SINUSITIS BY RHAMNOLIPIDS

FIELD OF THE INVENTION

This invention relates to methods of treating rhinitis and sinusitis by intranasal delivery of a rhamnolipid formulation.

BACKGROUND OF THE INVENTION

Rhinitis is a term describing the symptoms produced by nasal irritation or inflammation. Symptoms of rhinitis include runny nose, itching, sneezing and stuffy nose due to blockage or congestion. These symptoms are the nose's natural response to inflammation and irritation, and they are often associated with itching of the eyes.

The nose normally produces mucus, which traps substances like dust, pollen, pollution, and germs such as bacteria and viruses. Mucus flows from the front of the nose and drains down the back of the throat. When mucus production is excessive, it can flow from the front, as a runny nose, or become noticeable from the back, as post-nasal drip. Nasal mucus, normally a thin, clear liquid, can become thick or colored, perhaps due to dryness, infection or pollution. When post-nasal drip is excessive, thick, or contains irritating substances, cough is the natural response for clearing the throat.

Itching and sneezing are also natural responses to irritation caused by allergic reactions, chemical exposures including cigarette smoke, or temperature changes, infections and other factors.

The nasal tissues congest and decongest periodically. In most people, nasal congestion switches back and forth from side to side of the nose in a cycle several hours long. Some people, especially those with narrow nasal passages, notice this nasal cycle more than others. Severe congestion can result in facial pressure and pain, as well as dark circles under the eyes.

The three most common types of rhinitis are allergic, infectious, and non-allergic. Infectious rhinitis (colds or flu) are typically caused by viruses; its duration is often 3 to 7 days, and sometimes longer. Colds usually begin with a sensation of congestion, rapidly followed by runny nose and sneezing. Over the next few days, congestion becomes more prominent, the nasal mucus may become colored, and there may be a slight fever and cough. Cold symptoms resolve within a couple of weeks, although a cough may sometimes persist. Cold symptoms that last longer may be due to other causes, such as chronic rhinitis or

sinusitis.

Non-allergic rhinitis refers to rhinitis having symptoms not caused by infection or allergy. Non-allergic rhinitis includes vasomotor rhinitis and irritant rhinitis. Many people have recurrent or chronic nasal congestion, excess mucus production, itching, and other nasal symptoms similar to those of allergic rhinitis, but the disorder is not caused by allergy. Irritants that can trigger vasomotor rhinitis include cigarette smoke, strong odors and fumes including perfume, hair spray, cosmetics, laundry detergents, cleaning solutions, pool chlorine, car exhaust and other air pollution. Other irritants are spices used in cooking, alcoholic beverages (particularly beer and wine), aspirin, and certain blood pressure medications. Some people are very sensitive to abrupt changes in weather or temperature. Skiers often develop a runny nose, but in some people any cold exposure may cause a runny nose. Others start sneezing when leaving a cold, air conditioned room. The duration of symptoms of non-allergic rhinitis can be perennial and/or following exposure.

Allergic rhinitis, also known as hay fever, is often caused by dust mites, animals, pollens, molds, and food. Allergic rhinitis is an inflammatory state characterized by numerous symptoms such as nasal congestion, nasal discharge, post-nasal drip, sore throat, sneezing, headache, itching of the nose and throat, facial pressure and pain, and general malaise. Settipane et al (Ann. Allergy Asthma Immunol. 86:494-508 (2001)) report that allergic rhinitis is about 3 times more prevalent than pure nonallergic rhinitis; however, a mixed picture of the two is quite common: it is estimated that 44% to 87% of patients with rhinitis have some component of mixed rhinitis.

Perennial allergic rhinitis (PAR) is the most common type of allergic rhinitis, and is typically caused by exposure to allergens such as mold spores, dust mites, animal dander and others, and can occur at any time of year. This is generally viewed as a chronic disease. Seasonal allergic rhinitis, also known as hay fever, is a reaction to pollen or mold and typically occurs during certain seasons, for example during "rag weed season" in certain locals. The duration of the allergic reactions can be several days to a few months. Occupational allergic rhinitis is similar to PAR, but it is triggered by a response to airborne allergens at work. Infectious allergic rhinitis occurs during an upper respiratory infection, such as during the common cold, in which the infecting organism releases inflammatory mediators that trigger an allergic response. Symptoms last throughout the time of infection and are often associated with an increase in sinus and bronchial infections. Hormonal allergic rhinitis occurs typically during pregnancy or in patients with other hormonal imbalances such

as hypothyroidism. Idiopathic allergic rhinitis is a term used to describe allergic rhinitis in which either the allergen is not known or the cause of the inflammatory rhinitis symptoms is best defined as perennial non-allergic.

Allergic rhinitis clinically presents as some or all of the following symptoms: rhinorrhea, sneezing, nasal congestion, itching of the nose and palate, and ocular symptoms (itchy, watery eyes). (Skoner DP. Allergic rhinitis: definition, epidemiology, pathophysiology, detection, and diagnosis. *J Allergy Clin Immunol* 2001;108 (1 Suppl): S2-S8)

There is a close correlation between inflammatory mediators in nasal secretions and symptoms of Allergic Rhinitis. (Lebel B, Bousquet J, Morel A, et al. Correlation between symptoms and the threshold for release of mediators in nasal secretions during nasal challenge with grass-pollen grains. *J Allergy Clin Immunol* 1988;82(5 Pt 1):869-877)

Cytokines and chemokines, such as interleukin (IL)-8, as well as the mediators released by the early-phase reaction, help recruit and activate inflammatory cells, such as eosinophils, which themselves release mediators. (White M. Mediators of inflammation and the inflammatory process. *J Allergy Clin Immunol* 1999;103(3 Pt 2):S378-S381)

Symptoms are therefore perpetuated, with persistent allergic rhinitis sufferers existing in a continual state of eosinophilia and increased mediator release. (Wang DY, Clement P. Pathogenic mechanisms underlying the clinical symptoms of allergic rhinitis. *Am J Rhinol* 2000;14:325-333)

Eosinophils express various membrane molecules, including FcεRI. (Capron, M., Soussi Gounni, A., Morita, M., Truong, M.J.,Prin, L., Kinet, J.P. Capron, A. Eosinophils: from low- to high-affinity immunoglobulin E receptors. *Allergy* 1995, 50 (25 Suppl):20-23.)

Binding of the allergen-IgE complex with FcεRI on the eosinophil surface results in signal transduction, which activates the cell to release preformed, granule-associated proteins, arachidonic acid-derived products, cytokines and oxygen free radicals. (Capron, M., Desreumaux, P. Immunobiology of eosinophils in allergy and inflammation. *Res Immunol* 1997, 148:29-33)

A significant eosinophil activation may occur also in the very early events characterizing the reaction to allergen exposure. (Sihra, B.S., Kon, O.M., Grant, J.A., Kay, A.B. Expression of high-affinity IgE receptors (Fc epsilon RI) on peripheral blood basophils, monocytes and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations. *J Allergy Clin Immunol* 1997, 99:699-706.)

In addition to mast cells and eosinophils, epithelial cells are also activated after

allergen challenge. [Ciprandi, G., Pronzato, C., Ricca, V., Passalacqua, G., Bagnasco, M., Canonica, G.W. Allergen-specific challenge induces intercellular adhesion molecule 1 (ICAM-1 or CD54) on nasal epithelial cells in allergic subjects. Relationships with early and late inflammatory phenomena. *Am J Respir Crit Care Med* 1994, 150:1653-1659. Vignola, A.M., Campbell, A.M., Lacoste, P., Michel, F.B., Godard, P., Bousquet, J. Activation by histamine of bronchial epithelial cells from non asthmatic subjects. *Am J Respir Cell Mol Biol* 1993, 9:411-417.

Allergic rhinitis and asthma are closely-related entities influenced by common pathogenetic processes, linked by similar physiologic characteristics, sustained and amplified by interconnected mechanisms in atopic individuals. A recent clinical study has shown close correlations between bronchial reactivity and eosinophil percentages in nasal brushing, 'at baseline' and after nasal allergen challenge. (Silvestri M, Battistini E, Defilippi A-C, Sabatini F., Sale R, Pecora S, and Rossi GA. *J Invest Allergol Clin Immunol* 2005; Vol. 15(4): 266-276)

A recent study documented the role of CCL22/macrophage-derived chemokine (MDC) in allergic rhinitis. Dendritic cells (DCs) are considered to be the most powerful antigen-presenting cells. Expression of mRNA of dendritic cells using cDNA array and expression of CCL22/macrophage-derived chemokine (MDC) differed significantly between birch pollen allergic rhinitis and healthy controls. CCL22/MDC production was also higher in patients than in healthy donors. (Yanai M, Sato K, Aoki N, Takiyama Y, Oikawa K, Kobayashi H, Kimura S, Harabuchi Y, Tateno M. The role of CCL22/macrophage-derived chemokine in allergic rhinitis. Clin Immunol. 2007 Dec;125(3):291-8. Epub 2007 Oct 29.)

Biopsies taken from the nasal mucosa and in epithelial cell samples from 22 grass-pollen-allergic subjects before season, after allergen challenge and during season confirmed the presence of diverse macrophage subpopulations in the nasal mucosa of allergic subjects and indicated a central role of these cells not only in antigen processing but also in late phase reactions of allergic rhinitis. (Bachert C, Behrendt H, Nosbüsch K, Hauser U, Ganzer U. Possible role of macrophages in allergic rhinitis. Int. Arch. Allergy Appl. Immunol. (1991))

Allergic rhinitis is an inflammatory reaction, where a high degree of cell-to-cell communication is needed to orchestrate this inflammatory immune response. A variety of cytokines and adhesion receptors play an important role in the allergic late phase reaction. (C. Bachert, U. Hauser, B. Prem, C. Rudack and U. Ganzer. Proinflammatory cytokines in allergic rhinitis. European Archives of Oto-Rhino-Laryngology Volume 252, Supplement 1 /

January, 1995)

Chemoattractants such as IL-5 promote the infiltration of the mucosa with macrophages (and eosinophils, neutrophils, basophils, T lymphocytes). These cells become activated and release inflammatory mediators, and which reactivate many of the proinflammatory reactions of the early-phase response). (Naclerio RM, Proud D, Togias AG, et al. Inflammatory mediators in late antigen-induced rhinitis. N Engl J Med 1985; 313:65-70. Bascom R, Pipkorn U, Lichtenstein LM, Naclerio RM. The influx of inflammatory cells into nasal washings during the late response to antigen challenge: effect of systemic steroid pretreatment. Am Rev Respir Dis 1988; 138:406-412.)

Macrophages produce thymus and activation regulated chemokine (TARC), which are chemoattractants for lymphocytes, monocytes, eosinophils, basophils. [Sekiya T, Miyamasu M, Imanishi M, et al. Inducible expression of a TH2-type CC chemokine. J Immunol 2000; 165:2205-2213].

A pulmonary macrophage-monocyte-derived oligopeptide has been previously reported to induce mucus secretion in an in vitro model system with human airway explants and secretory epithelial cells. (Sperber K, Goswami SK, Gollub E, Mayer L, Marom Z. Mucus secretagogue production by a human macrophage hybridoma. J Allergy Clin Immunol. 1991 Feb;87(2):490-8.)

In moderate-to-severe allergic rhinitis, intranasal corticosteroids are first-line therapy. (Dykewicz MS, Fineman S, Skoner DP, et al. Diagnosis and management of rhinitis: complete guidelines of the Joint Task Force on Practice Parameters in Allergy, Asthma and Immunology. American Academy of Allergy, Asthma, and Immunology.Ann Allergy Asthma Immunol 1998;81(5 Pt 2):478-518)

When administered intranasally in therapeutic doses, corticosteroids have a direct anti-inflammatory action on the nasal mucosa, the mechanism of which is not yet completely defined. Corticosteroids are very effective. However, when allergic rhinitis symptoms are very severe, topical corticosteroids are not as effective as treatment with larger doses of oral or parenteral formulations with all the associated risk for diabetic patients and systemic immunosuppression such as reactivation of bacterial and viral infections. Furthermore, corticosteroids do not have an immediate effect on allergic signs and symptoms. Full benefit may be expected in 3 to 4 days. However, symptomatic relief may not occur in some patients for as long as two weeks. Corticosteroids have been associated with a number of local and systemic reactions, including epistaxis and nasal ulceration, nasal septal perforation and

impaired wound healing and also candida infection. Furthermore, they have been associated with glaucoma and cataracts. Patients should also be warned of potential worsening of existing bacterial, fungal or ocular herpes simplex infections. Adverse events reported during the post-marketing experience include: nasal discomfort and congestion, sneezing, alterations of taste and smell, nausea, insomnia, dizziness, fatigue, dyspnea, decreased blood cortisol, cataract, glaucoma, increased ocular pressure, pruritis, rash and hypersensitivity. Furthermore, controlled clinical studies have shown that intranasal corticosteroids may cause a reduction in growth velocity in pediatric patients. (Nasacort Product Monograph: www.sanofi-aventis.ca/products/en/nasacort%20aq.pdf)

Sinusitis (rhinosinusitis) is inflammation or infection of any of the four groups of sinus cavities in the skull, which open into the nasal passages. Sinusitis is not the same as rhinitis, although the two may be associated and their symptoms may be similar. Sinusitis affects approximately 16% of U.S. adults. Viral upper respiratory tract infections and allergic rhinitis, which affects up to 35.9 million Americans each year, are the two most common predisposing conditions for sinusitis. Medical treatments that are useful in treating recurrent acute and chronic sinusitis include the same range of medications as used for rhinitis, such as intranasal corticosteroids and decongestants.

Coexisting sources of nasal inflammation can manifest themselves with similar symptoms and can participate in the diffuse problem of sinusitis. There is histopathologic evidence that rhinitis was associated with chronic sinusitis. Several of the consensus documents mentioned above, most recently that published simultaneously in the Journal of Allergy and Clinical Immunology and Otolaryngology—Head and Neck Surgery in December 2004, officially adopted the term rhinosinusitis in preference to sinusitis. (Rhinosinitis and the revised "Sinusitis practice parameters". Hamilos DL et al. J Allergy and Clin Immunol. Dec. 2005)

Sleep apnea is a sleep disorder characterized by pauses in breathing during sleep. Each episode, called an apnea, lasts long enough so that one or more breaths are missed, and such episodes occur repeatedly throughout sleep. The standard definition of any apneic event includes a minimum 10 second interval between breaths, with either a neurological arousal, a blood oxygen desaturation of 3-4% or greater, or both arousal and desaturation. Sleep apnea is diagnosed with an overnight sleep test called a polysomnogram, or a "Sleep Study".

The individual with sleep apnea is rarely aware of having difficulty breathing, even upon awakening. Sleep apnea is recognized as a problem by others witnessing the individual

during episodes or is suspected because of its effects on the body (sequelae). Symptoms may be present for years (or even decades) without identification, during which time the sufferer may become conditioned to the daytime sleepiness and fatigue associated with significant levels of sleep disturbance.

Nasal resistance is an independent predictor of apnea-hypopnea index in nonobese obstructive sleep apnea patients. Rhinitis alone is associated with mild obstructive sleep apnea, but commonly causes microarousals and sleep fragmentation. (Staevska MT, Mandajieva MA, Dimitrov VD. Rhinitis and sleep apnea. Curr Allergy Asthma Rep. 2004 May;4(3):193-9.)

Nasal obstruction often leads to snoring and excessive daytime sleepiness. Hiraki et al. reported that when compared with a control group, the nasal obstruction group, independently of allergic rhinitis, had a greater percentage of snorers, a higher Epworth Sleepiness Scale score, and a greater percentage of participants with excessive daytime sleepiness. (Arch Otolaryngol Head Neck Surg. 2008;134:1254-1257) The odds of snoring and of having excessive daytime sleepiness were greater both in the nasal obstruction group with allergic rhinitis and the nasal obstruction group without allergic rhinitis. The authors assume that, although nasal obstruction itself is not a life-threatening condition, prompt and appropriate rhinologic treatment would improve sleep quality and, thus, daily and social activities in patients with sinonasal diseases.

Rhamnolipids are naturally occurring biosurfactants constructed of rhamnose sugar molecules and ß-hydroxyalkanoic acids. Production of rhamnose containing glycolipids was first described in Pseudomonas aeruginosa by Jarvis and Johnson. (Jarvis, F. G. and Johnson, M. J., A glycolipid produced by Pseudomonas aeruginosa. J. Am. Oil Chem. Soc., 1949, 71, 4124–4126.)

L-Rhamnosyl-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate and L-rhamnosyl-β-hydroxydecanoyl-β-hydrocydecanoate, are the principal glycolipids produced by P. aeruginosa. (Edward, J. R. and Hayashi, J. A., Structure of rhamnolipid from Pseudomonas aeruginosa. Arch. Biochem. Biophys., 1965, 111, 415–421.)

Studies have demonstrated the profound inhibitory effects of *P. aeruginosa* rhamnolipids on macrophage function. Sublytic concentrations of rhamnolipids have been shown to cause structural changes in human monocyte-derived macrophages. Rhamnolipids also inhibit in vitro phagocytosis by macrophages. Furthermore, internalization of attached particles and the level of phagosome-lysosome fusion of internalized targets within

macrophages is inhibited by rhamnolipids. (McClure CD and Schiller NL. Inhibition of Macrophage Phagocytosis by Pseudomonas aeruginosa Rhamnolipids In Vitro and In Vivo. Current Microbiology, Volume 33, Number 2 / August, 1996)

Rhamnolipids have demonstrated low irritancy and even anti-irritating effects when used topically on the skin. (Stipcevic T, et al. J Dermatol Sci. 2005 November; 40(2): 141–143.)

U.S. Patent No. 7,261,171 discloses the use of rhamnolipids in re-epithelization of skin, particularly in wound healing with the diminution of fibrosis.

U.S. Patent No. 5,514,661 discloses the use of rhamnolipids for treating autoimmune diseases of organ specific and organ non-specific autoimmune diseases, AIDS, Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis.

There is a need for improved methods for treating rhinitis, sinusitis, and sleep apnea due to upper airway obstruction. The method should be safe, effective and have no significant side effects.

SUMMARY OF THE INVENTION

The present invention is directed to a method for treating rhinitis or sinusitis in a subject. The method comprises the steps of: identifying a subject in need thereof, and administering intranasally to the subject a formulation comprising an effective amount of rhamnolipid, whereby the symptoms of the disease in the subject are reduced. The formulation preferably comprises rhamnolipid 1, rhamnolipid 2, or the combination.

The present invention is also directed to a method for treating sleep apnea due to upper airway obstruction. The method comprises the steps of: identifying a subject suffering from sleep apnea due to upper airway obstruction, and administering intranasally to the subject a formulation comprising an effective amount of rhamnolipid, whereby the symptoms in the subject are reduced. The formulation preferably comprises rhamnolipid 1, rhamnolipid 2, or the combination.

DETAILED DESCRIPTION OF THE INVENTION

The inventor has discovered that rhamnolipids, when used as a topical agent and intranasally administered to a person with allergic rhinitis, provide rapid and protracted alleviation of symptoms of allergic rhinitis, for up to 12 hours. The inventor also discovered that rhamnolipids, when used as a topical agent and intranasally administered to a patient,

improved rhinorrhea, nasal congestion, nasal itching, or sneezing.

The present invention is directed to a method for treating rhinitis. The method comprises the steps of: identifying a subject suffering from rhinitis, and administering intranasally to the subject a pharmaceutical formulation comprising an effective amount of rhamnolipid, whereby the symptoms of rhinitis in the subject are reduced. "The effective amount," as used herein, refers to an amount that alleviates or reduces one or more symptoms of rhinitis such as nasal congestion, nasal discharge, post-nasal drip, sore throat, sneezing, headache, itching of the nose and throat, facial pressure and pain, and general malaise.

The present invention is suitable to treat rhinitis including allergic rhinitis, infectious rhinitis, and non-allergic rhinitis. Allergic rhinitis is caused by allergens and affects more than 15 percent of adults and children. Allergic rhinitis includes seasonal allergic rhinitis (also called hay fever) and perennial allergic rhinitis. Seasonal allergic rhinitis is caused by outdoor allergens like pollen and mold. Perennial allergic rhinitis is caused by indoor allergens like animal dander and dust mites. Symptoms of allergic rhinitis include sneezing, runny nose, and nasal congestion. There may also be itching in the eyes, ears, nose and roof of the mouth.

Infectious rhinitis is caused by a common cold. Infections usually are self-limiting and subside after about a week. Infectious rhinitis symptoms that last longer may be due to a noninfectious rhinitis. Symptoms of infectious rhinitis include nasal congestion, runny nose, sneezing, fever and coughing.

Many people who suffer from rhinitis do not have an allergy or an infection. Nonallergic rhinitis has many triggers including smoke, cooking odors, spicy foods and certain medications. The cause of nonallergic rhinitis is still not fully understood, but the symptoms are similar to those of allergic rhinitis and sometimes include excess mucus production, congestion and itching.

The present invention is suitable to treat allergic rhinitis such as perennial, seasonal, and idiopathic allergic rhinitis. The present invention is also suitable to treat non-allergic rhinitis such as vasomotor, occupational, and hormonal rhinitis. The present invention is also suitable to treat mixed allergic rhinitis and non-allergic rhinitis.

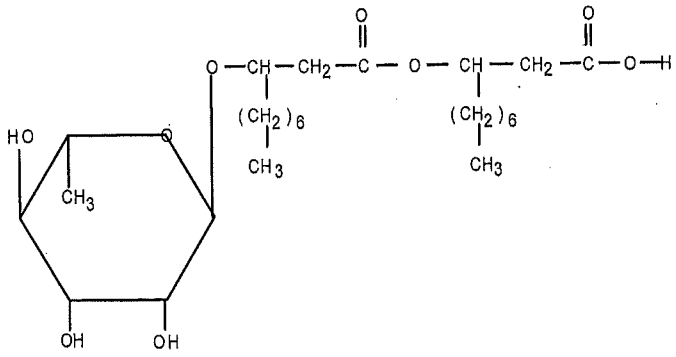
The present invention is also directed to a method for treating sinusitis. The method comprises the steps of: identifying a subject suffering from sinusitis, and administering intranasally to the subject a pharmaceutical formulation comprising an effective amount of rhamnolipid, whereby the symptoms of sinusitis in the subject are reduced. "The effective

amount," as used herein, refers to an amount that alleviates or reduces one or more symptoms of sinusitis such as nasal congestion, increased nasal secretions (rhinorrhea), nasal itching, sneezing.

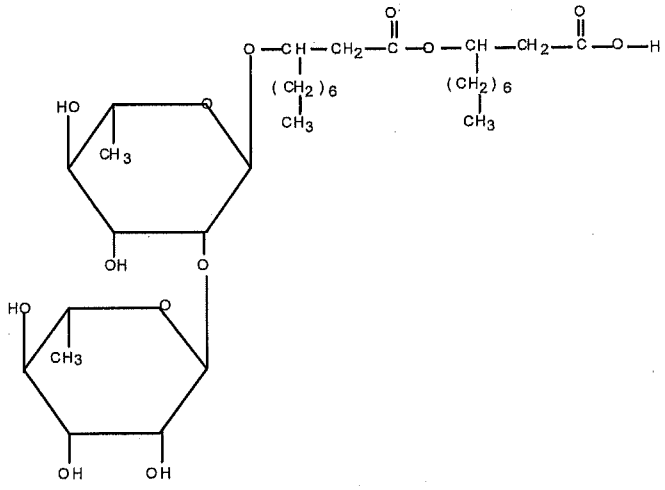
The present invention is also directed to a method for treating sleep apnea due to upper airway obstruction, for example, as a result of allergic rhinitis. The method comprises the steps of: identifying a subject suffering from sleep apnea due to upper airway obstruction, and administering intranasally to the subject a formulation comprising an effective amount of rhamnolipid, whereby the symptoms in the subject are reduced. "The effective amount," as used herein, refers to an amount that alleviates or reduces one or more symptoms of sleep apnea such as apneic events, snoring, and daytime sleepiness. The inventor has discovered that when the rhamnolipid formulation was applied every 12 hours for 14 days to a patient, the symptoms of sleep apnea was almost completely alleviated for up to 12 hours.

Rhamnolipids are biosurfactants containing rhamnose sugar molecules and β–hydroxyalkanoic acids. Rhamnolipids suitable to be used in the present invention include natural rhamnolipids, for example, obtained from Pseudomonas aeruginosa; rhamnolipids produced by any Pseudomonad, including P. chlororaphis, Burkholdera pseudomallei, Burkholdera (Pseudomonas) plantarii, and any recombinant Pseudomonad. Suitable rhamnolipids also includes those produced by other bacteria or by plants either naturally or through (genetic) manipulation. Suitable rhamnolipids further include rhamnolipids and their analogs prepared by chemical synthesis. Suitable rhamnolipids include those disclosed in U.S. Patent Nos. 7,262,171 and 5,514,661, in which the structures of rhamnolipids are incorporated herein by reference.

Preferred rhamnolipids are L-rhamnosyl-β-hydroxydecanoyl-β-hydrocydecanoate (rhamnolipids 1) and L-rhamnosyl-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (rhamnolipids 2), and the mixture thereof.



Rhamnolipid 1



Rhamnolipid 2 (Rha-Rha-C10-C10)

Some other common di-rhamnolipids useful for this invention include: L-rhamnopyranosyl-L-rhamnopyranosyl-beta-hydroxydecanoyl-beta-hydroxydodecanoate (often referred to as Rha-Rha-C10-C12); L-rhamnopyranosyl-L-rhamnopyranosyl-beta-hydroxytetradecanoyl-beta-hydroxytetradecanoate (often referred to as Rha-Rha-C14-C14).

The pharmaceutical formulation used for this invention typically contains pure rhamnolipid(s) and a pharmaceutically acceptable carrier in an aqueous solution. The concentration of rhamnolipid(s) is in general 0.005-10% (w/w) in a topical formulation, preferably 0.02-5 %, more preferably 0.1-5%, more preferably 0.2 to 5%, and most preferably 0.2-2% (w/w). In one embodiment, the concentration of rhamnolipid(s) is about 0.5% (w/w). "About" as used herein, refers to ± 15% of the recited value. The concentration can be higher or lower as long as it delivers an effective and tolerable dose per surface of nasal

mucosa per dosing interval. For example, a lower concentration can be used for irrigation, and a higher concentration can be used for inhalation.

Pharmaceutically acceptable carriers can be selected by those skilled in the art using conventional criteria. Pharmaceutically acceptable carriers include, but are not limited to, saline solution, aqueous electrolyte solutions, isotonicy modifiers, water polyethers such as polyethylene glycol, polyvinyls such as polyvinyl alcohol and povidone, cellulose derivatives such as methylcellulose and hydroxypropyl methylcellulose, polymers of acrylic acid such as carboxypolymethylene gel, polysaccharides such as dextrans, and glycosaminoglycans such as sodium hyaluronate and salts such as sodium chloride and potassium chloride.

In one embodiment, the pharmaceutical formulation of the present invention provides an aqueous solution comprising water, suitable ionic or non-ionic tonicity modifiers, suitable buffering agents, and rhamnolipids. In one embodiment, the rhamnolipid is at 0.005-10% (w/w), and the aqueous solution has a tonicity of 200-400 mOsm/kG and a pH of 4-9.

The pharmaceutical formulation is preferably formulated to have pH between 4.5-8, more preferably 5-7.4. The topical formulation may contain a buffer to facilitate a stable pH of 5-7.4.

The pharmaceutical formulation optionally contains non-ionic tonicity agents such as mannitol, sucrose, dextrose, glycerol, polyethylene glycol, propylene glycol, or ionic tonicity agent such as sodium chloride. The pharmaceutical formulation can further contains ionic or non-ionic surfactants, bile salts, phospholipids, cyclodextrins, micelles, liposomes, emulsions, polymeric microspheres, or their combination.

The pharmaceutical formulation is applied directly intra-nasally 1-3 times daily, preferably 1-2 times daily, until major symptoms of allergic rhinitis are eliminated. Then the frequency of applying the topical formulation is slowly reduced and is applied as required to alleviate symptoms of allergic rhinitis or sleep apnea due to upper airway obstruction.

Health regulations in various countries require that multi-dose nasal preparations include a preservative. Rhamnolipids have intrinsic antibacterial and antiviral properties, therefore limiting the need for preservatives. However, the topical formulation may contain commercially available preservation agents.

The pharmaceutical formulation is preferred to be stable at room temperature for at least 12 months, preferably 24 months, and more preferably 36 months. Stability, as used herein, means that rhamnolipid maintains at least 80%, preferably 85%, 90%, or 95% of its initial activity value.

The pharmaceutical formulations of the present invention can be prepared by aseptic technique. The purity levels of all materials used in the preparation preferably exceed 90%.

The pharmaceutical formulation of the present invention is administered locally to the nose in the form of nasal preparations. The pharmaceutical formulation can be administered to the nasal cavity of a patient topically by any suitable means, but is preferably administered in the form of drops or spray; with spray being more preferred. For topical nasal administration, one or two sprays per nostril of the formulation are delivered to the surface of the nose one to three times, preferably two times per day, according to the routine discretion of a skilled clinician. The pharmaceutical formulation can also be inhaled by the subject using a nebulizer.

The pharmaceutical formulation is preferably packaged in opaque plastic containers equipped with a nasal spray pump for topical nasal delivery.

The pharmaceutical formulation of the present invention can be used to prevent or treat diseases or disorders related to allergic and inflammatory diseases of the nose. For example, the pharmaceutical formulation is useful for treating seasonal and perennial allergic rhinitis, infectious rhinitis, vasomotor rhinitis, and sinusitis.

The following examples further illustrate the present invention. These examples are intended merely to be illustrative of the present invention and are not to be construed as being limiting.

EXAMPLES

Example 1

A deionized water solution of rhamnolipids (JBR 515, a mixture of R1 and R2) at 15% concentration was obtained from Jeneil Biosurfactant Co., LLC (Saukville, WI). The rhamnolipid solution was diluted in water to 0.5% (w/w) and applied to a subject that suffered from perennial rhinitis. The same 0.5% (w/w) rhamnolipid formulation was also used in Examples 5-10.

The 0.5% rhamnolipid formulation was applied to the subject. A standard Q-Tip was dipped into the 0.5% rhamnolipid formulation and then the Q-Tip was applied gently onto the intranasal areas of the subject; the subject's intranasal area had eschars due to excessive mechanical rubbing of the assessable intranasal areas with paper handkerchiefs for excessive itching and nasal discharge associated with his perennial rhinitis.

The symptoms of his allergic rhinitis (nasal congestion, nasal hypersecretion, and sneezing) disappeared almost immediately for approximately 4 hours.

After 4 hours on the day of the first administration, the subject re-administered the same amount of rhamnolipid solution into both nostrils at approximately 2200h, but as far into the intranasal space as was comfortable and swiped the intranasal surfaces that could be comfortably assessed. The subject repeated this on the opposite side, again using a standard Q-tip soaked with the 0.5 % rhamnolipid formulation. The subject then went to bed a short time later and noticed that, in contrast to his normal state, his nose was not congested and nasal passages were completely clear. The subject could breathe easily and was told that he did not snore, which was highly unusual for the subject.

The subject continued treatment with this same formulation twice daily for more than 14 days. Shortly prior to administration of a new dose of the above-mentioned formulation, the subject's symptoms of allergic rhinitis (especially nasal congestion, nasal hypersecretion, and sneezing) reappeared and then, subsequent to renewed administration of the formulation, the symptoms rapidly disappeared. His snoring has virtually ceased and he feels much more vigilant throughout the day while receiving rhamnolipid formulation as described above..

Example 2

Objective

The objective of this study is to compare the treatment effect of the invention in a cohort of 5-20 volunteers with a history of allergic rhinitis to their standard treatment.

Subjects

Subjects are 18-70 years of age with a history of allergic rhinitis (acute or perennial allergic rhinitis which are taking intra-nasally applied antihistamines or corticosteroids.

Test Protocols

Volunteers first have their TNSS (a standard rhinitis symptom score) established prior to their use of their standard medication and then again after approximately four hours.

The TNSS is the sum of four nasal symptom scores (runny nose, nasal congestion, itchy nose, and sneezing), each evaluated by the subject on a 0-3 scale. The TNSS can range from 0 to 12 total points.

Changes in TNSS before and after standard treatment and the difference in TNSS score

before and after rhamnolipid is compared.

Whenever the volunteers subsequently require their standard treatment after their initial dose of the usual intranasal treatment, the same volunteers receive a single dose of rhamnolipid formulation at a concentration of 0.25, 0.5, or 1%. Each dose administration is delivered as 1 – 2 swipes of the formulation applied either as drops or via a Q-Tip to all assessable intranasal surfaces.

Evaluation Criteria

TNSS scores prior to and at 4 hours after the administration of the standard treatment and then prior to and 4 hours after administration of rhamnolipid administration are assessed. After each dose administration, a questionnaire is completed for each subject documenting their symptom score prior to administration and then after 4 hours and any discomfort or undue sensations they may have observed that are different between the two administrations. The results are summarized in tabular form.

Example 3

Objective

The objective is to assess repeated doses of rhamnolipid on efficacy and morphological effects on mucosa of chronic administration of rhamnolipid formulation in a rodent model of allergic rhinitis.

An animal model involves a 7-14-day, randomized, double-blind comparison of repeated doses (1-5 doses per day, as guided by the response of the individual animals) of a dose of rhamnolipid nasal spray (0.25, 0.5% or 1.0% w/w) or placebo in rodents. Each group has 5-15 animals. The following or similar established model is used: Six- to 8-week-old BALB/c mice is sensitized by means of intranasal (local) application of ovalbumin (OVA) or systemic intraperitoneal injection. The animals are then challenged with intranasal OVA, and allergic response is assessed.

Efficacy of Treatment

The primary endpoints of the trial are histological and immunological changes in nasal mucosa samples. Histology of the upper and lower airways assess degree of cellular infiltration into mucous and tissue.

Results of the trial are assessed using standard statistical methods to depict trends and analyze

responses to determine any statistical differences between placebo and rhamnolipid group's outcomes. Significance of any improvement is analyzed ($p<0.05$) of the rhamnolipid dose groups compared to placebo.

Example 4

Objective

To evaluate the efficacy of rhamnolipid in treating rhinitis in an animal model.

Material

Animal:

Female BALB-c mice each weighing 20-30 grams were used in this study.

Sensitization:

The followings are used to sensitize the mice:

1. 0.5 ml saline (0.9% sodium chloride), containing 25 μg of OVA (grade V, Sigma Chemical Co., St. Louis, MO, USA) and 2mg of aluminum hydroxide (alum) (pH 5.5, 308 mOsmol L^{-1}) were used as intraperitoneal injection;
2. 5 μl of 5% ovalbumin solution in saline was instilled in each nostril. Isoflurane (an anesthetic agent) was given prior to the intranasal administrations.

Rhamnolipids:

5 μl of 0.5% rhamnolipids (ABI-100, a mixture of rhamnolipid 1 and rhamnolipid 2) in saline was administered for each nostril.

Method

Mice were sensitized by 5 weekly intraperitoneal injection of ovalbumin (at Day 1, 8, 15, 22, and 29). After the 5 weekly intraperitoneal injections, from Day 32-50), mice were given 19 once-daily intranasal doses of ovalbumin (5 μl of 5% ovalbumin solution in saline instilled in each nostril). The mice were anesthetized using isoflurane prior to the intranasal instillation of ovalbumin.

On Day 49, 20 mice were assigned to a treatment group and a placebo group. The mice in the treatment group received 5 μl of a 0.5% rhamnolipid mixture in saline per nostril. This intranasal dose was administered twice, with the doses administered 1 hour apart.

Mice were observed for signs of nasal irritation for a 15 minute continuous evaluation period, on the day of treatment (Day 49), and the day following treatment (Day 50). Half of the mice were also evaluated two days after treatment (Day 51). The investigators were blinded to the identity of the agent, i.e., which mice had received saline and which mice had received active treatment.

The number of sneezes and nose rubbings were counted. Nose rubbings were only counted if both paws were employed, and multiple rubbings in one quick succession were counted as one nose-rubbing event. The total (symptom) score was defined as the total number of sneezes and nose rubbings.

Results

The means, standard deviations (SD), and standard errors (SE) of total (symptom) score in the placebo and treatment groups are recorded in Table 1.

Table 1

	Ovalbumin-sensitized animals treated with Saline (Placebo Group)				Ovalbumin-sensitized animals treated with Rhamnolipid (Treatment Group)			
	No. of Mice	Total Score Mean	SD	SE	No. of Mice	Total Score Mean	SD	SE
Day 1 after Treatment	10	13.9	5.6	1.8	10	8.9	3.4	1.1

Day 1 after treatment

One hour after renewed intranasal instillation of the OVA allergen on the first day after rhamnolipid application (Day 50), the average total symptom score in the treatment group was 8.9, compared to 13.9 in the control group. The difference in the total scores between the placebo and treatment groups was statistically significant (P =0.0269).

Conclusions:

This study shows rhamnolipids were effective in treating allergic rhinitis in a mouse

model. Rhamnolipids resulted in a statistically significant reduction of total rhinitis symptom score in the treatment group when compared with the control group one day post-treatment.

Example 5

The 0.5% (w/w) rhamnolipid formulation (same as that described in Example 1) was applied to a healthy male, 66 years old, who had a history of mild nasal congestion. The rhamnolipid formulation was applied to the subject as 2 intranasal administrations, separated by 5 minutes, using Q-Tips soaked in the rhamnolipid formulation (a new Q-tip for every application). Each Q-Tip was dipped into the 0.5% rhamnolipid formulation and then applied by the subject gently onto the intranasal surfaces of the subject. The subject reported within minutes of dosing that his nasal passages were clearer, and this feeling lasted for 4 hours.

Example 6

The 0.5% (w/w) rhamnolipid formulation was applied to a healthy male (48 years of age), who had no history of allergic rhinitis, and had a history of mild nasal congestion prior to application. The 0.5% rhamnolipid formulation was applied to the subject as 2 intranasal administrations, separated by 5 minutes, using Q-Tips soaked in the rhamnolipid formulation (a new Q-tip for every application). Each Q-Tip was dipped into the 0.5% rhamnolipid formulation and then applied by the subject gently onto the intranasal surfaces of the subject.

Upon application, the subject reported a slight tingling (more noticeable after administration), 'medicinal' smell, 'moistening' sensation; all those symptoms subsided within 1-2 minutes. He noted that subsequently both nasal passages were more open than usual. One hour post-dose he noticed remarkably open nasal passages ('the clearest they've ever been'), and this sensation lasted until 18 hours post-dose.

Example 7

A healthy 61 year old male, with no pertinent medical history, specifically no allergic rhinitis, noted that beginning symptoms of a 'head-cold' might have started. The subject noticed a scratchy throat but nothing else, specifically: no rhinorrhea, no nasal congestion, itching or sneezing. The 0.5% rhamnolipid formulation was applied to the subject as 2 intranasal administrations, separated by 5 minutes, using Q-Tips soaked in the rhamnolipid formulation (a new Q-tip for every application). Each Q-Tip was dipped into the 0.5%

rhamnolipid formulation and then applied by the subject gently onto the intranasal surfaces of the subject.

Subsequent to self-application of the rhamnolipid solution, the subject described a 'soothing and mentholated' sensation. The subject did not notice any tingling, any form of local irritation, rhinorrhea, nasal congestion, itching or sneezing. One hour after dosing, the subject noted that his nasal passages were more open than usual and this feeling lasted for 17 hours post-dosing and he experienced no rhinorrhea, no nasal congestion, itching or sneezing. The symptom of head-cold was still confined to throat: no 'scratchiness', but productive cough.

Example 8

A 49 year old female with recurrent sinusitis (three events that required treatment within the 2 months preceding rhamnolipid application) self-applied rhamnolipid topically. She complained of moderate rhinorrhea and severe nasal congestion. The 0.5% rhamnolipid formulation (same as that described in Example 1) was applied to the subject as 2 intranasal administrations, separated by 5 minutes, using Q-Tips soaked in the rhamnolipid formulation (a new Q-tip for every application). Each Q-Tip was dipped into the 0.5% rhamnolipid formulation and then applied by the subject gently onto the intranasal surfaces of the subject. This was repeated twice daily for ten days.

By the second day of rhamnolipid application, the subject reported a significant improvement of her symptoms. By day 3, the subject reported virtually complete alleviation of symptoms of sinusitis that lasted during the entire course of rhamnolipid application and even 3 days beyond the date at which rhamnolipid application was stopped.

Example 9

The subject was a 54 year old male that smoked at least 30 cigarettes per day and was described as a heavy snorer by his spouse. He reported mild rhinorrhea and moderate nasal congestion (symptoms of non-allergic rhinitis), particularly at night. He self-applied a 0.5% rhamnolipid formulation as 2 intranasal administrations, separated by 5 minutes, using Q-Tips soaked in the rhamnolipid formulation (a new Q-tip for every application). Each Q-Tip was dipped into the 0.5% rhamnolipid formulation and then applied by the subject gently onto the intranasal surfaces of the subject. This was repeated twice daily for 14 days.

By the second day of application, the symptoms of rhinorrhea and nasal congestion were completely alleviated. By day 7 he, according to his spouse, the subject virtually stopped snoring. These effects continued for 3 days after succession of application.

Example 10

A 54 year old female with mild rhinorrhea and moderate nasal congestion (suspected non-allergic rhinitis) was described as a moderate snorer by her spouse. She self-applied a 0.5% rhamnolipid formulation as 2 intranasal administrations, separated by 5 minutes, using Q-Tips soaked in the rhamnolipid formulation (a new Q-tip for every application). Each Q-Tip was dipped into the 0.5% rhamnolipid formulation and then applied by the subject gently onto the intranasal surfaces of the subject. This was repeated twice daily for 14 days.

By the evening of the second day of application, the symptoms of rhinorrhea and nasal congestion were completely alleviated in the subject. Further, according to her spouse, the subject virtually stopped snoring. The subject noted that she awakened much more refreshed than usual from a night's sleep. Although the subject had symptoms of a common cold on day 7, she did not experience any nasal congestion as she usually would have, and her general symptoms of the common cold subsided after 2 days. After completion of the 14 days of rhamnolipid application, the alleviation of signs of mild rhinorrhea, moderate nasal congestion, and snoring continued for 3 days.

Example 11

A 24 year old male with a long history of allergic rhinitis with severe congestion and mild rhinorrhea self-applied a 0.5% rhamnolipid formulation as 2 intranasal administrations, separated by 5 minutes, using Q-Tips soaked in the rhamnolipid formulation (a new Q-tip for every application). Each Q-Tip was dipped into the 0.5% rhamnolipid formulation and then applied by the subject gently onto the intranasal surfaces of the subject. This was a single dose application. Symptoms of congestion were almost totally alleviated within 4 hours, with a concomitant transient increase in rhinorrhea. In the evening of the day of application, the subject complained of somewhat greater than normal congestion that improved during the night. Beginning the next day, his nose was totally free for the first time in months and this lasted for approximately 36 hours.

The invention, and the manner and process of making and using it, are now described in such full, clear, concise and exact terms as to enable any person skilled in the art to which it

pertains, to make and use the same. It is to be understood that the foregoing describes preferred embodiments of the present invention and that modifications may be made therein without departing from the scope of the present invention as set forth in the claims. To particularly point out and distinctly claim the subject matter regarded as invention, the following claims conclude the specification.

WHAT IS CLAIMED IS:

1. A method for treating rhinitis in a subject, comprising the steps of:

identifying a subject suffering from rhinitis;

administering intranasally to the subject a formulation comprising an effective amount of rhamnolipid, whereby the symptoms of rhinitis in the subject are reduced.

2. The method according to Claim 1, wherein the rhinitis is allergic rhinitis, infectious rhinitis, or non-allergic rhinitis.

3. The method according to Claim 2, wherein the allergic rhinitis is perennial allergic rhinitis or seasonal allergic rhinitis.

4. The method according to Claim 2, wherein the non-allergic rhinitis is vasomotor rhinitis.

5. The method according to Claim 1, wherein the rhinitis is mixed allergic rhinitis and non-allergic rhinitis.

6. A method for treating sinusitis in a subject, comprising the steps of:

identifying a subject suffering from sinusitis;

administering intranasally to the subject a formulation comprising an effective amount of rhamnolipid, whereby the symptoms of sinusitis in the subject are reduced.

7. The method according to any one of Claims 1-6, wherein said rhamnolipid is selected from the group consisting of: rhamnolipid 1, rhamnolipid 2, and the combination thereof.

8. The method according to Claim 7, wherein said effective amount is 0.1-5% (w/w).

9. The method according to Claim 7, wherein said effective amount is 0.2-2% (w/w).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/68111

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/70; A01N 43/04 (2010.01)
USPC - 514/25
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 31/70; A01N 43/04 (2010.01)
USPC - 514/25

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/9, 514/25, 514/54 (words only)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
USPTO PubWEST (PGPB, USPT, USOC, EPAB, JPAB); Google
Search Terms: Rhinitis, runny nose, rhamnolipid, intranasal, intranasally, sinusitis, vasomotor and ciliostatic factor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2005/0031549 A1 (QUAY, et al.) 10 February 2005, especially, para [0012], [0029], [0032], [0120], [0387], [0427]	1-3 --- 4-9
Y	US 7,378,082 B1 (KRISHNAMOORTHY) 27 May 2008 (27.05.2008), especially, col 7, ln 26-31	4-6
Y	Rhamnolipid, Inc. Rhamnolipid Basics. Published 19 April 2008, pg 1 [online], document retrieved on 25 January 2010, [retrieved from http://web.archive.org/web/20080419011148/www.rhamnolipid.com/rhamnolipidbasics.html], especially, pg 1, para 3.	7-9

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 January 2010 (25.01.2010)	19 FEB 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)