METHODS OF TREATING A PULMONARY BACTERIAL INFECTION USING FLUOROQUINOLONES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 61/172,625, filed April 24, 2009. The entire priority document is hereby incorporated by reference in its entirety.

BACKGROUND

Field

[0002] This application relates to the fields of pharmaceutical chemistry and medicine. In particular, it relates to methods of treating pulmonary bacterial infections.

Description of the Related Art

[0003] The pathogen associated with most chronic infections affecting cystic fibrosis (CF) patients is *Pseudomonas aeruginosa*. According to the Cystic Fibrosis Foundation (CFF), approximately 55% of CF patients are colonized with *P. aeruginosa*. Severe pulmonary exacerbations are a common manifestation from chronic *P. aeruginosa* infections.

[0004] *P. aeruginosa* can grow under anaerobic conditions by using nitrate or nitrite for anaerobic respiration, or by fermentation of arginine. Sputum from CF patients contain average nitrate levels of 250-350 μM and can contain levels as high as 1000 μM. Therefore, CF sputum can provide *P. aeruginosa* cells with an environment in which to promote and sustain colonization under anaerobic conditions.

[0005] It has been demonstrated that areas of low oxygen tension exist within dense pulmonary secretions in the lungs of CF patients. Although typically aerobic, *P. aeruginosa* can colonize and proliferate within these microaerophilic environments in CF sputum.

SUMMARY

[0006] Some embodiments disclosed herein relate to methods of treating a pulmonary bacterial infection including administering a therapeutically effective amount of an aerosol of a fluoroquinolone antibiotic, wherein the pulmonary bacterial infection includes bacteria capable of growing under anaerobic conditions.

[0007] Some embodiments include a method of treating a pulmonary bacterial infection including administering a therapeutically effective amount of an aerosol of a fluoroquinolone antibiotic selected from the group consisting of levofloxacin and ofloxacin, wherein the pulmonary bacterial infection includes bacteria growing under anaerobic conditions.

[0008] In some embodiments, the method includes assaying the pulmonary bacterial infection for the presence of bacteria growing under anaerobic conditions. The bacteria, in some embodiments, are growing under anaerobic conditions using nitrate or nitrite.

[0009] Some embodiments further include assaying the pulmonary bacterial infection for the presence of bacteria growing under anaerobic conditions using nitrate or nitrite. In some embodiments, the bacteria include Pseudomonas aeruginosa. In some embodiments, the method includes assaying the pulmonary bacterial infection for the presence of Pseudomonas aeruginosa.

[0010] The fluoroquinolone antibiotic, in some embodiments, is levofloxacin. The fluoroquinolone antibiotic, in some embodiments, is ofloxacin.

[0011] In some embodiments, at least a portion of the pulmonary bacterial infection is growing under anaerobic conditions. In some embodiments, the pulmonary bacterial infection is identified as having at least a portion of said bacteria growing under anaerobic conditions.

[0012] Some embodiments have the pulmonary bacterial infection in a subject with cystic fibrosis. Some embodiments have the pulmonary infection characterized by sputum including nitrate levels of at least 250 μM. In some embodiments, pulmonary bacterial infection is identified as having sputum comprising nitrate levels of at least 250 μM.

[0013] In some embodiments, the method of treating the pulmonary bacterial infection does not include administering a therapeutically effective amount of an antibiotic selected from the group consisting of tobramycin, amikacin and aztreonam.

[0014] In some embodiments, no other antibiotics are administered in a therapeutically effective amount to treat the pulmonary bacterial infection. In some embodiments, the fluoroquinolone antibiotic is administered by intrapulmonary delivery. In some embodiments, the therapeutically effective amount of fluoroquinolone is more

than about 5 mg. In some embodiments, the therapeutically effective amount of fluoroquinolone os no more than about 150 mg.

[0015] Some embodiments have a method of inhibiting bacteria growing under anaerobic conditions comprising exposing said bacteria to an amount of a fluoroquinolone antibiotic effective to inhibit the growth of said bacteria.

[0016] The bacteria, in some embodiments, is exposed to a mixture comprising at least about 0.75 mg/L of the fluoroquinolone antibiotic. In some embodiments, the bacteria include Pseudomonas aeruginosa. In some embodiments, the bacteria is identified as growing under anaerobic conditions.

[0017] Some embodiments of the method include assaying a sample of said bacteria to determine if the bacteria is growing under anaerobic conditions. In some embodiments, a sample of the bacteria is characterized by nitrate levels of at least 250 μM.

[0018] The fluoroquinolone antibiotic, in some embodiments, is levofloxacin. The fluoroquinolone antibiotic, in some embodiments, is ofloxacin.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **FIG. 1** is a table showing aerobic and anaerobic MIC testing of various antimicrobials.

[0020] **FIG. 2A** is a graph of the *P. aeruginosa* aerobic and anaerobic MIC distributions of levofloxacin (LVX).

[0021] **FIG. 2B** is a graph of the *P. aerations* aerobic and anaerobic MIC distributions of tobramycin (TOB).

[0022] **FIG. 2C** is a graph of the *P. aeruginosa* aerobic and anaerobic MIC distributions of amikacin (AMK).

[0023] **FIG. 2D** is a graph of the *P. aeruginosa* aerobic and anaerobic MIC distributions of aztreonam (ATM).

[0024] **FIG. 3A** is a graph of the mean log CFU/mL of *P. aeruginosa* over time for the strain PAM1020, wild-type.

[0025] **FIG. 3B** is a graph of the mean log CFU/mL of *P. aeruginosa* over time for the strain PAM1032, *na1B*.

[0026] **FIG. 3C** is a graph of the mean log CFU/mL of *P. aeruginosa* over time for the strain PAM1481, *na1B gyrA*.

[0027] **FIG. 3D** is a graph of the mean log CFU/mL of *P. aeruginosa* over time for the strain PAM1573, *na1B gyrA* (Thr83I1e).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0028] Cystic fibrosis is a hereditary disease that results in frequent pulmonary bacterial infections requiring treatment with antibiotics. U.S. Publication No. 2006/0276483, which is hereby incorporated by reference in its entirety, teaches aerosolized fluoroquinolones and their uses for treating bacterial pulmonary infections.

[0029] Some types of bacteria that may be present in a pulmonary bacterial infection can grow under anaerobic conditions. It has been demonstrated that areas of low oxygen tension exist within dense pulmonary secretions in the lungs of CF patients. Thus, a pulmonary bacterial infection may have bacteria growing under anaerobic conditions. Hypoxic environments, which can be found in CF patients, can impede the potency of some classes of antibiotics and therefore improved methods of treatment are necessary.

[0030] Surprisingly, it has been found that fluoroquinolones exhibit similar activity against bacteria growing in both aerobic and anaerobic conditions.

Definitions

[0031] The term "microbe" refers to microscopic organisms, such bacteria or fungi. Thus, any disclosure of this term also contemplates features relating to the narrower class of "bacteria." For example, descriptions relating to antimicrobial compounds also contemplate using antibiotics.

[0032] The term "administration" or "administering" refers to a method of giving a dosage of an antimicrobial pharmaceutical composition to a vertebrate. The preferred method of administration can vary depending on various factors, e.g., the components of the pharmaceutical composition, the site of the potential or actual bacterial infection, the microbe involved, and the severity of an actual microbial infection.

[0033] The term "mammal" is used in its usual biological sense. Thus, it specifically includes humans, cattle, horses, dogs, and cats, but also includes many other species.

[0034] The term "microbial infection" refers to the undesired proliferation or presence of invasion of pathogenic microbes in a host organism. This includes the excessive growth of microbes that are normally present in or on the body of a mammal or other organism. More generally, a microbial infection can be any situation in which the presence of a microbial population(s) is damaging to a host mammal. Thus, a microbial

infection exists when excessive numbers of a microbial population are present in or on a mammal's body, or when the effects of the presence of a microbial population(s) is damaging the cells or other tissue of a mammal.

[0035] In the context of the response of a microbe, such as a bacterium, to an antimicrobial agent, the term "susceptibility" refers to the sensitivity of the microbe for the presence of the antimicrobial agent. So, to increase the susceptibility means that the microbe will be inhibited by a lower concentration of the antimicrobial agent in the medium surrounding the microbial cells. This is equivalent to saying that the microbe is more sensitive to the antimicrobial agent. In most cases the minimum inhibitory concentration (MIC) of that antimicrobial agent will have been reduced.

[0036] By "therapeutically effective amount" or "pharmaceutically effective amount" is meant an amount of fluoroquinolone antimicrobial agent, which has a therapeutic effect. The doses of fluoroquinolone antimicrobial agent which are useful in treatment are therapeutically effective amounts. Thus, as used herein, a therapeutically effective amount means those amounts of fluoroquinolone antimicrobial agent which produce the desired therapeutic effect as judged by clinical trial results and/or model animal infection studies. In particular embodiments, the fluoroquinolone antimicrobial agent are administered in a pre-determined dose, and thus a therapeutically effective amount would be an amount of the dose administered. This amount and the amount of the fluoroquinolone antimicrobial agent can be routinely determined by one of skill in the art, and will vary, depending on several factors, such as the particular microbial strain involved. This amount can further depend upon the patient's height, weight, sex, age and medical history. For prophylactic treatments, a therapeutically effective amount is that amount which would be effective to prevent a microbial infection.

[0037] A "therapeutic effect" relieves, to some extent, one or more of the symptoms of the infection, and includes curing an infection. "Curing" means that the symptoms of active infection are eliminated, including the total or substantial elimination of excessive members of viable microbe of those involved in the infection to a point at or below the threshold of detection by traditional measurements. However, certain long-term or permanent effects of the infection may exist even after a cure is obtained (such as extensive tissue damage). As used herein, a "therapeutic effect" is defined as a statistically significant reduction in bacterial load in a host, emergence of resistance, or improvement in infection symptoms as measured by human clinical results or animal studies.

[0038] "Treat," "treatment," or "treating," as used herein refers to administering a pharmaceutical composition for prophylactic and/or therapeutic purposes. The term "prophylactic treatment" refers to treating a patient who is not yet infected, but who is susceptible to, or otherwise at risk of, a particular infection. The term "therapeutic treatment" refers to administering treatment to a patient already suffering from an infection. Thus, in preferred embodiments, treating is the administration to a mammal (either for therapeutic or prophylactic purposes) of therapeutically effective amounts of a fluoroquinolone antimicrobial agent.

Method of Treatment

[0039] Some embodiments disclosed herein are methods of treating a pulmonary bacterial infection that include administering a therapeutically effective amount of an aerosol of a fluoroquinolone antimicrobial, wherein the pulmonary bacterial infection comprises bacteria growing under anaerobic conditions.

[0040] The therapeutically effective amount may include, for example, at least about 5 mg; at least about 10 mg; at least about 20 mg; or at least about 50 mg. Similarly, therapeutically effective amount may include, for example, no more than about 150 mg; no more than about 140 mg; no more than about 125 mg; or no more than about 100 mg.

[0041] Because fluoroquinolones exhibit activity against bacteria growing under anaerobic conditions, the method may include assaying the pulmonary bacteria infection for the presence of bacteria growing, or capable of growing, under anaerobic conditions. For example, a culture may be taken of the infection and the type of bacteria present determined. If there are bacteria capable of growing under anaerobic conditions, a treatment including administering a fluoroquinolone can be used. Moreover, using such an assay, other criteria may be used to determine if a treatment including administering a fluoroquinolone is appropriate. A fluoroquinolone may be appropriate when there are bacteria growing under anaerobic condition using a nitrite or nitrate, or alternatively, when the bacteria is *Pseudomonas aeruginosa*.

[0042] Various fluoroquinolones may be used to treat the pulmonary bacterial infections. In an embodiment, the fluoroquinolone is selected form the group consisting of levofloxacin and ofloxacin. In another embodiment, the fluoroquinolone can be levofloxacin. In still another embodiment the fluoroquinolone can be ofloxacin. The fluoroquinolones can be in aerosol form to allow intrapulmonary delivery.

[0043] In some embodiments, the method does not include treating the pulmonary bacterial infection with a therapeutically effective amount of tobramycin, amikacin or aztreonam. In another embodiment, no other antimicrobials are administered in a therapeutically effective amount to treat the pulmonary bacterial infection.

[0044] The type of pulmonary infections to be treated is not particularly limited. The pulmonary infection may include an infection found in a patient with cystic fibrosis. Also, the method may be used to treat a pulmonary bacterial infection that is characterized by sputum comprising average nitrate levels of at least about 250 μM or at least about 500 μM. Finally, the method may be used for a pulmonary bacterial infection that has at least a portion of the bacteria growing under anaerobic conditions.

[0045] Moreover, various types of bacteria for treatment are contemplated, so long as the bacteria are growing, or capable of growing, under anaerobic conditions. For example, the bacteria may be *Pseudomonas aeruginosa*. In an embodiment, the treatment includes bacteria growing, or capable of growing, under anaerobic conditions using nitrate or nitrite.

EXAMPLES

[0046] Embodiments of the present application are disclosed in further detail in the following examples, which are not in any way intended to limit the scope of the invention.

Bacterial strains and antibiotics

[0047] One hundred and fourteen CF *P. aeruginosa* isolates were obtained for susceptibility testing from the CF Referral Center for Susceptibility & Synergy Studies at Columbia University (New York, NY) and also from two CF Therapeutics Development Network (TDN) laboratories (Seattle Children's Hospital, Seattle, WA and University of North Carolina at Chapel Hill, Chapel Hill, NC). About sixty percent were recent isolates (2004-2007) with the remaining forty percent isolated between 1980 and 2004.

[0048] *P. aeruginosa* strains PAM1020 (wild-type), PAM1032 (*nalB*), PAM1481 (*nalB gyrA* (*Asp87Tyr*)), and PAM1573 (*nalB gyrA* (Thr83Ile)) represent relevant efflux-mediated and target mutation resistance mechanisms and were used in the levofloxacin time-kill assays.

[0049] The antibiotics used in these studies included tobramycin, levofloxacin, amikacin, and aztreonam which are in use or in development as aerosolized therapies for CF. For aerobic susceptibility tests, levofloxacin hydrochloride, tobramycin sulfate, and

amikacin disulfate were purchased from LKT Laboratories (St. Paul, MN) and aztreonam base was purchased from MP Biomedicals (Solon, OH). All antibiotics used for anaerobic susceptibility tests were purchased from the United States Pharmacopeia (Rockville, MD).

Susceptibility testing

[0050] Antibiotic MIC endpoints were obtained using the broth microdilution method according to the CLSI reference method. See Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically - Seventh Edition: Approved Standard M7-A7. CLSI, Wayne, PA, USA, 2006. Antibiotics were serially diluted to the following concentrations for aerobic testing: levofloxacin and tobramycin from 0.03 - 32 mg/L and amikacin and aztreonam from 0.125 - 128 mg/L. Anaerobic susceptibility testing required the addition of 1% potassium nitrate (KNO_3) to cation-adjusted Mueller-Hinton broth (CAMHB) to allow for *P. aeruginosa* anaerobic respiration. For anaerobic testing, frozen MIC plates were thawed and stored in the anaerobic chamber overnight to ensure elimination of all oxygen prior to inoculation with test strains. The dilution range for all antibiotics was 0.125 - 128 mg/L for the anaerobic susceptibility tests. Extended incubation of up to 48 hours under anaerobic conditions may be required and was needed for 54% of the isolates.

Bactericidal activity

[0051] Aerobic and hypoxic time-kill assays were conducted to determine the bactericidal activity of levofloxacin at concentrations ranging from 32 – 1,024 mg/L. Levofloxacin concentrations ranged from 16-fold to 2,048-fold the MIC against isogenic *P. aeruginosa* strains PAM1020 (MIC = 0.125 mg/L), PAM1032 (MIC = 1 mg/L), PAM1481 (MIC = 4 mg/L) and PAM1573 (MIC = 8 mg/L). Aerobic and hypoxic Mueller-Hinton broth (MHB) cultures were diluted to an initial inoculum of $1 \times 10^7 - 1 \times 10^8$ CFU/ml. Hypoxic conditions were simulated by maximizing the MHB volume in the growth vessel and omitting shaking during incubation at 37°C. Growth rates using these conditions or MHB treated with the Oxyrase enzyme system (Oxyrase, Inc., Mansfield, OH) were similar. The final culture volume was 10 ml. At 0, 10, 20, 40, 80 and 160 minutes, 0.5 ml samples were removed from each culture, immediately washed twice with MHB to minimize levofloxacin carryover effects, serially diluted with physiologic saline and plated on Mueller-Hinton agar (MHA). Agar plates were incubated up to 48 hours at

37°C and bactericidal activity was assessed. The limit of detection was 2 $\log_{10}$ CFU/ml. Bacterial counts obtained following incubation under either condition were compared using a paired t-test.

Results

[0052] The results for aerobic and anaerobic MIC testing are summarized in the table shown in FIG. 1. There was little change in the potency of levofloxacin under anaerobic conditions; the MIC_{50} only increased 2-fold, with no increase in MIC_{90}. In contrast, anaerobic incubation increased the geometric means of the MIC for tobramycin, amikacin, and aztreonam by approximately 7-fold, 4-fold, and 6-fold, respectively, with MIC_{50} values for tobramycin and aztreonam increasing 4- and 16-fold, respectively under anaerobic conditions. More than 40% of the isolates had MICs increase >4-fold to tobramycin, amikacin and aztreonam compared to only 4% for levofloxacin.

[0053] FIGS. 2A-D show the distribution of aerobic and anaerobic MIC results for each antibiotic with all 114 *P.* aeruginosa isolates. Under anaerobic conditions, tobramycin, amikacin, and aztreonam demonstrated reduced potency, indicated by the shift in MIC distribution. In contrast, the aerobic and anaerobic MIC distributions for levofloxacin were similar.

[0054] Time-kill curves were developed to determine the bactericidal activity of high concentrations of levofloxacin attained following aerosol administration against isogenic *P. aeruginosa* strains under aerobic and hypoxic conditions to simulate the partial oxygen gradient present in the lungs of CF patients. Rapid and sustained in vitro bactericidal activity within 10 minutes was observed for each strain at each levofloxacin concentration under both conditions ($p>0.05$), as shown in FIG. 3.

WHAT IS CLAIMED IS:

1. Use of a therapeutically effective amount of an aerosol of a fluoroquinolone antibiotic selected from the group consisting of levofloxacin and ofloxacin in the manufacture of a medicament for the treatment of pulmonary Pseudomonas aeruginosa bacterial infection in a subject in need thereof, wherein the aerosol is for intrapulmonary use and wherein the pulmonary bacterial infection comprises Pseudomonas aeruginosa bacteria growing under anaerobic conditions, and where the bacteria is exposed to 0.125-1,024 mg/L of the fluoroquinolone antibiotic.

2. The use of Claim 1, wherein the pulmonary bacterial infection is assayed for the presence of bacteria growing under anaerobic conditions prior to use of the fluoroquinolone antibiotic.

3. The use of Claim 1 or 2, wherein the bacteria is capable of growing under anaerobic conditions using nitrate or nitrite.

4. The use of Claim 3, wherein the pulmonary bacterial infection is assayed for the presence of bacteria capable of growing under anaerobic conditions using nitrate or nitrite prior to use of the fluoroquinolone antibiotic.

5. The use of Claim 1, wherein the pulmonary bacterial infection is assayed for the presence of Pseudomonas aeruginosa prior to use of the fluoroquinolone antibiotic.

6. The use of any one of Claims 1-5, wherein the fluoroquinolone antibiotic is levofloxacin.

7. The use of any one of Claims 1-5, wherein the fluoroquinolone antibiotic is ofloxacin.

8. The use of any one of Claims 1-7, wherein said pulmonary bacterial infection is identified as having at least a portion of said bacteria growing under anaerobic conditions.

9. The use of any one of Claims 1-8, wherein the pulmonary bacterial infection is in a subject with cystic fibrosis.

10. The use of any one of Claims 1-9, wherein the pulmonary bacterial infection is characterized by sputum comprising nitrate levels of at least 250 μM.

11. The use of any one of Claims 1-10, wherein the pulmonary bacterial infection is identified as having sputum comprising nitrate levels of at least 500 μM.

12. The use of any one of Claims 1-11, where said therapeutically effective amount of fluoroquinolone is more than about 5 mg.

13. The use of any one of Claims 1-11, where said therapeutically effective amount of fluoroquinolone is no more than about 150 mg.

14. Use of a mixture comprising a pharmaceutically acceptable carrier and at least 0.75 mg/L of a fluoroquinolone antibiotic selected from levofloxacin and ofloxacin to inhibit Pseudomonas aeruginosa bacteria growing under anaerobic conditions.

15. The use of Claim 14, wherein a sample of said bacteria is assayed to determine if said bacteria is growing under anaerobic conditions, prior to use of the fluoroquinolone antibiotic.

16. The use of any one of Claims 14-15, wherein a sample of the bacteria is characterized by nitrate levels of at least 250 μM.

17. The use of any one of Claims 14-16, wherein the fluoroquinolone antibiotic is levofloxacin.

18. The use of any one of Claims 14-16, wherein the fluoroquinolone antibiotic is ofloxacin.