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(54) Title: CO-ADMINISTRATION OF MINOCYCLINE AND COLISTIN TO REDUCE ACUTE KIDNEY INJURY

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Disclosed herein are methods of treating bacterial infection using a combination of minocycline and colistin to reduce the likelihood of acute kidney injury.

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(54) Title: CO-ADMINISTRATION OF MINOCYCLINE AND COLISTIN TO REDUCE ACUTE KIDNEY INJURY

(57) Abstract: Disclosed herein are methods of treating bacterial infection using a combination of minocycline and colistin to reduce the likelihood of acute kidney injury.

CO-ADMINISTRATION OF MINOCYCLINE AND COLISTIN TO REDUCE ACUTE KIDNEY INJURY

BACKGROUND

Field

[0001] The present application relates to the fields of pharmaceutical chemistry, biochemistry and medicine. More specifically, the present application relates to pharmaceutical compositions, methods, and kits for treating subjects with bacterial infection using minocycline in combination with colistin to reduce acute kidney injury, and optionally in subjects at increased risk of kidney damage.

Description of the Related Art

[0002] Bacterial infections are contagious and may result in many serious or life-threatening complications. Antibiotics have been effective tools in combating bacterial infections for during the last half-century. Some bacterial infections are particularly problematic and resistant to treatment. Gram-negative bacteria cause infections including pneumonia, bloodstream infections, and wound infections. However, many gram-negative bacteria are resistant to multiple antibiotics available on the market. In such instances, last-resort antibiotics may be used to treat the bacterial infection, but these compounds can have many unwanted and often severe side effects.

[0003] Colistin is a decades-old drug that was first isolated in Japan in 1949 and became available from clinical use in 1959. Kumazawa, J., *et al*, *J. Infect. Chemother.* 2002, 8:125. However, colistin fell out of favor because of its significant nephro- and neurotoxicity. Wolinsky, E. *et al.*, *N. Engl. J. Med.* 1962, 266(15): 759–68; Koch-Weser J., *et al., Annals of Internal Medicine.* 1970, 72(6): 857–68. As multi-drug resistant bacteria became more prevalent in the 1990's, colistin started to get a second look as a drug of last resort despite its toxic effects.

[0004] Treating severe bacterial infections with nephrotoxic antibiotics as colistin may prove to be problematic even in healthy subjects, and particularly dangerous in subjects that are already susceptible to acute kidney injury or have diminished kidney function.

Accordingly, there exists a particular need for methods of treating bacterial infections with antibiotic agents that reduce the risk of acute kidney injury in a subject.

SUMMARY

[0005] Some embodiments disclose methods of treating bacterial infection in a subject, comprising administering to the subject minocycline in combination with colistin, thereby reducing the likelihood of acute kidney injury.

[0006] Some embodiments disclose methods of treating bacterial infection in a subject susceptible to acute kidney injury by administering to the subject a combination of minocycline and colistin. In some embodiments, the subject with the bacterial infection is also suffering from one or more additional conditions. In some embodiments, the subject is suffering from diabetes. In some embodiments, the subject is suffering from renal disease. In some embodiments, the subject is suffering from cancer.

[0007] Some embodiments disclose methods of treating bacterial infection in a subject having kidney function parameters indicating decreased kidney function by administering to the subject a combination of minocycline and colistin. In some embodiments, the subject has a glomerular filtration rate that is below normal. In some embodiments, the subject has an elevated urine albumin level. In some embodiments, the subject has an elevated serum creatinine level. In some embodiments, the subject has a creatinine clearance rate that is below normal. In some embodiments, the subject has an elevated blood urea nitrogen level.

[0008] In some embodiments disclosed herein, the subject may be suffering from one or more bacterial infections. In one embodiment, the subject may be infected with *Escherichia coli*. In one embodiment, the subject may be infected with *Klebsiella pneumoniae*. In one embodiment, the subject may be infected with *Pseudomonas aeruginosa*. In one embodiment, the subject may be infected with *Streptococcus pyogenes*. In one embodiment, the subject may be infected with *Streptococcus pneumoniae*. In one embodiment, the subject may be infected with *Haemophilus influenzae*. In one embodiment, the subject may be infected with *Chlamydia trachomatis*. In one embodiment, the subject may be infected with *Mycoplasma pneumoniae*. In one embodiment, the subject may be infected with *Legionella pneumophila*. In one embodiment, the subject may be infected with

Acinetobacter baumannii. In one embodiment, the subject may be infected with *Bartonella bacilliformis*. In one embodiment, the subject may be infected with *Brucella species*. In one embodiment, the subject may be infected with *Calymmatobacterium granulomatis*. In one embodiment, the subject may be infected with *Campylobacter fetus*. In one embodiment, the subject may be infected with *Francisella tularensis*. In one embodiment, the subject may be infected with *Haemophilus ducreyi*. In one embodiment, the subject may be infected with *Vibrio cholerae*. In one embodiment, the subject may be infected with and *Yersinia pestis*.

[0009] Some embodiments disclose a pharmaceutical composition comprising minocycline and colistin. In some embodiments, the composition may be formulated for oral administration. In some embodiments, the composition may be formulated for intravenous administration. In some embodiments, the composition may be formulated for intraperitoneal administration. In some embodiments, the composition may be formulated for intragastric administration. In some embodiments, the composition may be formulated for intravascular administration.

[0010] Some embodiments of the present invention described herein include kits comprising minocycline, colistin or combination thereof.

DETAILED DESCRIPTION

Definitions

[0011] As used herein, a "subject" refers to an animal that is the object of treatment, observation or experiment. "Animal" includes cold- and warm-blooded vertebrates and invertebrates such as fish, shellfish, reptiles, and, in particular, mammals. "Mammal" includes, without limitation, mice; rats; rabbits; guinea pigs; dogs; cats; sheep; goats; cows; horses; primates, such as monkeys, chimpanzees, and apes, and, in particular, humans.

[0012] As used herein, a "patient" refers to a subject that is being treated by a medical professional, such as a Medical Doctor (*i.e.* Doctor of Allopathic medicine or Doctor of Osteopathic medicine) or a Doctor of Veterinary Medicine, to attempt to cure, or at least ameliorate the effects of, a particular disease or disorder or to prevent the disease or disorder from occurring in the first place.

[0013] As used herein, "administration" or "administering" refers to a method of giving a dosage of a pharmaceutically active ingredient to a vertebrate.

[0014] As used herein, a "dosage" refers to an amount of therapeutic agent administered to a patient.

[0015] As used herein, a "daily dosage" refers to the total amount of therapeutic agent administered to a patient in a day.

[0016] As used herein, the term "therapeutic agent" means a substance that is effective in the treatment of a disease or condition.

[0017] As used herein, "therapeutically effective amount" or "pharmaceutically effective amount" is meant an amount of therapeutic agent, which has a therapeutic effect. The dosages of a pharmaceutically active ingredient which are useful in treatment are therapeutically effective amounts. Thus, as used herein, a therapeutically effective amount means those amounts of therapeutic agent which produce the desired therapeutic effect as judged by clinical trial results and/or model animal studies.

[0018] As used herein, a "therapeutic effect" relieves, to some extent, one or more of the symptoms of a disease or disorder. For example, a therapeutic effect may be observed by a reduction of the subjective discomfort that is communicated by a subject (*e.g.*, reduced discomfort noted in self-administered patient questionnaire).

Treatment of Bacterial Infection

[0019] In some embodiments disclosed herein, the subject may be suffering from one or more bacterial infections. In some embodiments, the subject may be infected with a gram-negative bacteria. In one embodiment, the subject may be infected with *Escherichia coli*. In one embodiment, the subject may be infected with *Klebsiella pneumoniae*. In one embodiment, the subject may be infected with *Pseudomonas aeruginosa*. In one embodiment, the subject may be infected with *Streptococcus pyogenes*. In one embodiment, the subject may be infected with *Streptococcus pneumoniae*. In one embodiment, the subject may be infected with *Haemophilus influenzae*. In one embodiment, the subject may be infected with *Chlamydia trachomatis*. In one embodiment, the subject may be infected with *Mycoplasma pneumoniae*. In one embodiment, the subject may be infected with *Legionella*

pneumophila. In one embodiment, the subject may be infected with *Acinetobacter baumannii*. In one embodiment, the subject may be infected with *Bartonella bacilliformis*. In one embodiment, the subject may be infected with *Brucella species*. In one embodiment, the subject may be infected with *Calymmatobacterium granulomatis*. In one embodiment, the subject may be infected with *Campylobacter fetus*. In one embodiment, the subject may be infected with *Francisella tularensis*. In one embodiment, the subject may be infected with *Haemophilus ducreyi*. In one embodiment, the subject may be infected with *Vibrio cholerae*. In one embodiment, the subject may be infected with *Yersinia pestis*.

[0020] In some embodiments, minocycline is co-administered with colistin to a subject to treat a bacterial infection. In some embodiments, minocycline is co-administered with colistin to a subject susceptible to acute kidney injury. In some embodiments, minocycline is co-administered with colistin to treat a bacterial infection in a subject suffering from one or more conditions selected from myocardial infarction, congestive heart failure, peripheral vascular disease, coronary artery disease, chronic pulmonary disease, diabetes, dementia, peptic ulcer disease, renal disease, cancer, liver disease, paraplegia, hemiplegia, HIV/AIDS, and rheumatic disease. In some embodiments, the co-administration of minocycline with colistin to a subject reduces the risk of acute kidney injury in the subject.

[0021] In some embodiments, the susceptibility of a subject to acute kidney injury may be indicated by one or more of the following kidney function parameters selected from, but not limited to: glomelular filtration rate (GFR), urine albumin level, serum creatinine level, creatinine clearance rate, and blood urea nitrogen level. Additionally, the susceptibility of a subject to acute kidney injury may be measured by ultrasound, computed tomography (CT), and/or magnetic resonance imaging (MRI).

[0022] The glomerular filtration rate (GFR) is a measure of how well the kidneys are removing wastes and excess fluids from the blood. Normal GFR may vary according to age. The normal value for GFR is 90 mL/min/1.73 m^2 or above, while a GFR below 60 is a sign that the kidneys are not functioning properly. A GFR below 15 is usually indicative of kidney failure and suggests that a subject will need dialysis or a kidney transplant.

[0023] In some embodiments, minocycline is co-administered with colistin to a subject having a GFR that may indicate decreased kidney function. In some embodiments, minocycline is co-administered with colistin to a subject having a GFR below about 120, about 110, about 100, about 90, about 80, about 70, about 60, about 50, about 40, about 30, about 20, or about 15 mL/min/1.73 m^2. In some embodiments, In some embodiments, minocycline is co-administered with colistin to a subject having a GFR in the range of about 15 to about 30 mL/min/1.73 m^2, about 20 to about 40 mL/min/1.73 m^2, about 15 to about 40 mL/min/1.73 m^2, about 40 to about 60 mL/min/1.73 m^2, about 30 to about 50 mL/min/1.73 m^2, about 30 to about 60 mL/min/1.73 m^2, about 50 to about 70 mL/min/1.73 m^2, about 50 to about 80 mL/min/1.73 m^2, about 50 to about 90 mL/min/1.73 m^2, about 60 to about 90 mL/min/1.73 m^2, about 70 to about 90 mL/min/1.73 m^2, or about 70 to about 100 mL/min/1.73 m^2.

[0024] The urine albumin level of a subject may be indicative of kidney damage. Albumin is a protein found in the blood, and a healthy kidney does not let albumin pass into the urine. A urine albumin level of below 30 mg per day is considered normal, whereas a urine albumin level above 30 mg per day is abnormal and may indicate kidney damage.

[0025] In some embodiments, the kidney function of a subject may be determined by measuring the subject's urine albumin level. In some embodiments, minocycline is co-administered with colistin to a subject having a urine albumin level above about 20, about 30, about 40, about 50, about 60, about 70, about 80, about 100, about 150, about 200, about 250, or about 300 mg per day. In some embodiments, minocycline is co-administered with colistin to a subject having a urine albumin level in the range of about 15 to about 30 mg per day, about 30 to about 50 mg per day, about 30 to about 60 mg per day, about 50 to about 100 mg per day, about 70 to about 100 mg per day, about 30 to about 150 mg per day, about 30 to about 300 mg per day, about 150 to about 200 mg per day, or about 150 to about 300 mg per day.

[0026] Creatinine is a waste product from the normal breakdown of muscle tissue. As creatinine is produced, it is filtered through the kidneys and excreted in urine. The creatinine clearance rate may be used to assess kidney health. Creatinine clearance in a

healthy young person is about 125 mL per minute and normally decreases with increasing age. A low creatinine clearance can demonstrate kidney injury.

[0027] In some embodiments, the kidney function of a subject may be determined by measuring the subject's creatinine clearance rate. In some embodiments, minocycline is co-administered with colistin to a subject having a creatinine clearance rate below about 140, about 130, about 120, about 100, about 90, about 80, about 70, about 60, about 50, about 40, about 30, or about 20 mL per minute. In some embodiments, minocycline is co-administered with colistin to a subject having a creatinine clearance rate in the range of about 20 to about 40 mL per minute, about 20 to about 60 mL per minute, about 30 to about 60 mL per minute, about 50 to about 70 mL per minute, about 20 to about 80 mL per minute, about 20 to about 90 mL per minute, about 50 to about 90 mL per minute, about 90 to about 110 mL per minute, or about 100 to about 140 mL per minute.

[0028] A subject's serum creatinine level indicates the level of creatinine in the blood. If the kidneys are not functioning properly, an increased level of creatinine level may accumulate in the blood. A normal serum creatinine level is about 0.7 to 1.3 mg/dL for men and about 0.6 to 1.1 mg/dL for women.

[0029] In some embodiments, the kidney function of a subject may be determined by measuring the subject's serum creatinine level. In some embodiments, minocycline is co-administered with colistin to a subject having a serum creatinine level abot about 0.5, about 0.6, about 0.7, about 0.8, about 0.9, about 1.0, about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 2.0 about 2.5, about 3.0 mL, about 4.0, or about 5.0 mg/dL. In some embodiments, minocycline is co-administered with colistin to a subject having a creatinine clearance rate in the range of about 0.7 to about 1.3 mg/dL, about 0.6 to about 1.1 mg/dL, about 1.0 to about 5.0 mg/dL, about 1.5 to about 4.0 mg/dL, about 2.0 to about 5.0 mg/dL, about 2.0 to about 4.0 mg/dL, or about 1.5 to about 5.0 mg/dL.

[0030] A blood urea nitrogen test is used to measure the amount of nitrogen in the subject's blood that comes from urea, a waste product that is made when protein is broken down from the body. A blood urea nitrogen test is done to see how well the kidneys are working. Normally, if the kidneys have impaired ability to remove urea from the blood urea

nitrogen level rises. Blood urea nitrogen tests are often used to determine if kidneys are functioning normally or if kidney disease is progressing.

[0031] In some embodiments, the kidney function of a subject may be determined by measuring the subject's blood urea nitrogen level. In some embodiments, minocycline is co-administered with colistin to a subject having a blood urea nitrogen level above about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, or about 60 mg/dL. In some embodiments, minocycline is co-administered with colistin to a subject having a blood urea nitrogen in the range of about 5 to about 20 mg/dL, about 10 to about 20 mg/dL, about 20 to about 30 mg/dL, about 20 to about 40 mg/dL, about 40 to about 50 mg/dL, or about 40 to about 60 mg/dL.

[0032] In some embodiments, minocycline and colistin are co-administered to a subject having decreased kidney function. While not being bound by any particular theory, in some embodiments, the co-administration of minocycline and colistin results in reducing the likelihood of acute kidney injury to a subject as compared to treatment with either minocycline or colistin alone.

[0033] The minocycline and colistin may be administered separately or in a single dosage form. In some embodiments, the minocycline is present and/or administered as a free base. In some embodiments, the minocycline is administered as a pharmaceutically acceptable salt. In some embodiments, the minocycline is administered as a hydrochloride salt. In some embodiments, colistin is is present and/or administered as a free base. In some embodiments, the colistin is administered as a pharmaceutically acceptable salt. In some embodiments, the colistin is administered as colistin sulfate. In some embodiments, the colistin is administered as colistimethate sodium. In one embodiment, the combination is administered as the minocycline hydrochloride salt and colistin sulfate salt. In one embodiment, the combination is administered as the minocycline hydrochloride salt and colistimethate sodium.

Pharmaceutical Compositions

[0034] In another aspect, the present disclosure relates to a pharmaceutical composition comprising a physiologically acceptable surface active agents, carriers, diluents,

excipients, smoothing agents, suspension agents, film forming substances, and coating assistants, or a combination thereof; and a compound disclosed herein. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, PA (1990), which is incorporated herein by reference in its entirety. Preservatives, stabilizers, dyes, sweeteners, fragrances, flavoring agents, and the like may be provided in the pharmaceutical composition. For example, sodium benzoate, ascorbic acid and esters of p-hydroxybenzoic acid may be added as preservatives. In addition, antioxidants and suspending agents may be used. In various embodiments, alcohols, esters, sulfated aliphatic alcohols, and the like may be used as surface active agents; sucrose, glucose, lactose, starch, crystallized cellulose, mannitol, light anhydrous silicate, magnesium aluminate, magnesium methasilicate aluminate, synthetic aluminum silicate, calcium carbonate, sodium acid carbonate, calcium hydrogen phosphate, calcium carboxymethyl cellulose, and the like may be used as excipients; magnesium stearate, talc, hardened oil and the like may be used as smoothing agents; coconut oil, olive oil, sesame oil, peanut oil, soya may be used as suspension agents or lubricants; cellulose acetate phthalate as a derivative of a carbohydrate such as cellulose or sugar, or methylacetate-methacrylate copolymer as a derivative of polyvinyl may be used as suspension agents; and plasticizers such as ester phthalates and the like may be used as suspension agents.

[0035] The minocycline and/or colistin can be formulated for administration with a pharmaceutically acceptable carrier or diluent. The minocycline and/or colistin can be formulated as a medicament with a standard pharmaceutically acceptable carrier(s) and/or excipient(s) as is routine in the pharmaceutical art. The exact nature of the formulation will depend upon several factors including the desired route of administration. Typically, minocycline and/or colistin are formulated for oral, inhalation, intravenous, intragastric, intravascular or intraperitoneal administration.

[0036] The term “pharmaceutical composition” refers to a mixture of a compound or compounds disclosed herein with other chemical components, such as diluents or carriers. The pharmaceutical composition facilitates administration of the compound(s) to an organism. Multiple techniques of administering a compound exist in the art including, but

not limited to, oral, injection, aerosol, parenteral, and topical administration. Pharmaceutical compositions can also be obtained by reacting compound(s) with inorganic or organic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

[0037] The term "carrier" defines a chemical compound that facilitates the incorporation of a compound into cells or tissues. For example dimethyl sulfoxide (DMSO) is a commonly utilized carrier as it facilitates the uptake of many organic compounds into the cells or tissues of an organism.

[0038] The term "diluent" defines a chemical compound diluted in water that will dissolve the compound of interest as well as stabilize the biologically active form of the compound. Salts dissolved in buffered solutions are utilized as diluents in the art. One commonly used buffered solution is phosphate buffered saline because it mimics the salt conditions of human blood. Since buffer salts can control the pH of a solution at low concentrations, a buffered diluent rarely modifies the biological activity of a compound.

[0039] The term "physiologically acceptable" defines a carrier or diluent that does not abrogate the biological activity and properties of the compound.

[0040] The pharmaceutical compositions described herein can be administered to a human patient *per se*, or in pharmaceutical compositions where they are mixed with other active ingredients, as in combination therapy, or suitable carriers or excipient(s). Techniques for formulation and administration of the compound or combination of compounds disclosed herein may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, 18th edition, 1990.

[0041] Some embodiments provide the compound(s) or combination of compounds disclosed herein in tablets, film coated tablets, capsules, caplets, pills, gel caps, pellets, beads, or dragée dosage forms. Preferably, the formulations disclosed herein can provide favorable drug processing qualities, including, for example, but not limited to, rapid tablet press speeds, reduced compression force, reduced ejection forces, blend uniformity, content uniformity, uniform dispersal of color, accelerated disintegration time, rapid dissolution, low friability (preferable for downstream processing such as packaging, shipping,

pick-and-pack, etc.) and dosage form physical characteristics (e.g., weight, hardness, thickness, friability) with little variation.

[0042] The compound(s) or combination of compounds disclosed herein can be formulated readily, for example, by combining the drug substance with any suitable pharmaceutically acceptable excipient(s) for example, but not limited to, binders, diluents, disintegrants, lubricants, fillers, carriers, coatings, glidants, flavours, color additives, and the like, as set forth below. Such compositions can be prepared for storage and for subsequent processing.

Excipients

[0043] Acceptable excipients for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Handbook of Pharmaceutical Excipients, 5th edition (Raymond C Rowe, Paul J Sheskey and Siân C Owen, eds. 2005), and Remington: The Science and Practice of Pharmacy, 21st edition (Lippincott Williams & Wilkins, 2005), each of which is hereby incorporated in its entirety. The term "carrier" material or "excipient" herein can mean any substance, not itself a therapeutic agent, used as a carrier and/or diluent and/or adjuvant, or vehicle for delivery of a therapeutic agent to a subject or added to a pharmaceutical composition to improve its handling or storage properties or to permit or facilitate formation of a dose unit of the composition into a discrete article such as a capsule, tablet, film coated tablet, caplet, gel cap, pill, pellet, bead, and the like suitable for oral administration. Excipients can include, by way of illustration and not limitation, diluents, disintegrants, binding agents, wetting agents, polymers, lubricants, glidants, coatings, sweetens, solubilizing agents, substances added to mask or counteract a disagreeable taste or odor, flavors, colorants, fragrances, and substances added to improve appearance of the composition.

[0044] The compositions and formulations can include any other agents that provide improved transfer, delivery, tolerance, and the like. These compositions and formulations can include, for example, powders, pastes, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LipofectinTM), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene

glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax.

[0045] Any of the foregoing mixtures can be appropriate in treatments and therapies in accordance with the disclosure herein, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance." Regul. Toxicol. Pharmacol. 32(2):210-8 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." J. Pharm. Sci. 89(8):967-78 (2000), and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[0046] In some embodiments, one or more, or any combination of the listed excipients can be specifically included or excluded from the formulations and/or methods disclosed herein. As will be appreciated by those of skill in the art, the amounts of excipients will be determined by drug dosage and dosage form size.

Lubricants

[0047] In some embodiments, lubricants are employed in the manufacture of certain dosage forms. For example, a lubricant will often be employed when producing tablets. In some embodiments, a lubricant can be added just before the tableting step, and can be mixed with the formulation for a minimum period of time to obtain good dispersal. In some embodiments, one or more lubricants can be used. Examples of suitable lubricants include, but are not limited to, magnesium stearate, calcium stearate, zinc stearate, stearic acid, talc, glyceryl behenate, polyethylene glycol, polyethylene oxide polymers (for example, available under the registered trademarks of Carbowax® for polyethylene glycol and Polyox® for polyethylene oxide from Dow Chemical Company, Midland, Mich.), sodium lauryl sulfate, magnesium lauryl sulfate, sodium oleate, sodium stearyl fumarate, DL-leucine, colloidal silica, and others as known in the art. Typical lubricants are magnesium stearate, calcium stearate, zinc stearate and mixtures of magnesium stearate with sodium lauryl sulfate.

Color Additives

[0048] In some embodiments, color additives also can be included. The colorants can be used in amounts sufficient to distinguish dosage form strengths. Preferably, color additives approved for use in drugs (21 CFR 74, which is incorporated herein by reference in its entirety) are added to the commercial formulations to differentiate tablet strengths. The use of other pharmaceutically acceptable colorants and combinations thereof are encompassed by the current disclosure.

Binders

[0049] Binders can be used, for example, to impart cohesive qualities to a formulation, and thus ensure that the resulting dosage form remains intact after compaction. Suitable binder materials include, but are not limited to, microcrystalline cellulose, gelatin, sugars (including, for example, sucrose, glucose, dextrose and maltodextrin), polyethylene glycol, waxes, natural and synthetic gums, polyvinylpyrrolidone, pregelatinized starch, povidone, cellulosic polymers (including, for example, hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), methyl cellulose, hydroxyethyl cellulose, and the like), hydroxypropyl cellulose (HPC), and the like. Accordingly, in some embodiments, the formulations disclosed herein can include at least one binder to enhance the compressibility of the major excipient(s). In some embodiments, the binder(s) is(are) sprayed on from solution, e.g. wet granulation, to increase binding activity.

Disintegrants

[0050] In some embodiments, disintegrants are used, for example, to facilitate tablet disintegration after administration, and are generally starches, clays, celluloses, algins, gums, or crosslinked polymers. Suitable disintegrants include, but are not limited to, crosslinked polyvinylpyrrolidone (PVP-XL), sodium starch glycolate, alginic acid, methacrylic acid DYB, microcrystalline cellulose, crospovidone, polacriline potassium, sodium starch glycolate, starch, pregelatinized starch, croscarmellose sodium, and the like. If desired, the pharmaceutical formulation can also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, sodium lauryl sulfate, dioctyl sodium sulfosuccinate, polyoxyethylene sorbitan fatty acid esters, etc. and the like.

Coatings

[0051] In some embodiments, the formulations can include a coating, for example, a film coating. Where film coatings are involved, coating preparations can include, for example, a film-forming polymer, a plasticizer, or the like. Also, the coatings can include pigments and/or opacifiers. Non-limiting examples of film-forming polymers include hydroxypropyl methylcellulose, hydroxypropyl cellulose, methylcellulose, polyvinyl pyrrolidine, and starches. Non-limiting examples of plasticizers include polyethylene glycol, tributyl citrate, dibutyl sebecate, castor oil, and acetylated monoglyceride. Furthermore, non-limiting examples of pigments and opacifiers include iron oxides of various colors, lake dyes of many colors, titanium dioxide, and the like.

Diluents

[0052] In some embodiments, diluents are used, and are generally selected from one or more of the compounds sucrose, fructose, glucose, galactose, lactose, maltose, invert sugar, calcium carbonate, lactose, starch, microcrystalline cellulose, lactose monohydrate, calcium hydrogen phosphate, anhydrous calcium hydrogen phosphate, a pharmaceutically acceptable polyol such as xylitol, sorbitol, maltitol, mannitol, isomalt and glycerol, polydextrose, starch, or the like, or any mixture thereof.

Surfactants

[0053] In some embodiments, surfactants are used. The use of surfactants as wetting agents in oral drug forms is described in the literature, for example in H. Sucker, P. Fuchs, P. Speiser, Pharmazeutische Technologie, 2nd edition, Thieme 1989, page 260. It is known from other papers, such as published in Advanced Drug Delivery Reviews (1997), 23, pages 163-183, that it is also possible to use surfactants, inter alia, to improve the permeation and bioavailability of pharmaceutical active compounds. Examples of surfactants include, but are not limited to, anionic surfactants, non-ionic surfactants, zwitterionic surfactants and a mixture thereof. Preferably, the surfactants is selected from the group consisting of poly(oxyethylene) sorbitan fatty acid ester, poly(oxyethylene) stearate, poly(oxyethylene) alkyl ether, polyglycolated glyceride, poly(oxyethylene) castor oil, sorbitan fatty acid ester, poloxamer, fatty acid salt, bile salt, alkyl sulfate, lecithin, mixed micelle of bile salt and

lecithin, glucose ester vitamin E TPGS (D-α-tocopheryl polyethylene glycol 1000 succinate), sodium lauryl sulfate, and the like, and a mixture thereof.

Glidants

[0054] In some embodiments, glidants are used. Examples of glidants which may be used include, but are not limited to, colloidal silicon dioxide, magnesium trisilicate, powdered cellulose, starch, talc and calcium phosphate, or the like, and mixtures thereof.

[0055] Suitable routes of administration may, for example, include oral, rectal, transmucosal, topical, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intravenous, intramedullary injections, as well as intrathecal, direct intraventricular, intraperitoneal, intranasal, or intraocular injections. The compound or combination of compounds disclosed herein can also be administered in sustained or controlled release dosage forms, including depot injections, osmotic pumps, pills, transdermal (including electrotransport) patches, and the like, for prolonged and/or timed, pulsed administration at a predetermined rate.

[0056] The pharmaceutical compositions of the present disclosure may be manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or tabletting processes.

[0057] Pharmaceutical compositions for use in accordance with the present disclosure thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. Any of the well-known techniques, carriers, and excipients may be used as suitable and as understood in the art; *e.g.*, in Remington's Pharmaceutical Sciences, above.

[0058] Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, cysteine hydrochloride, and the like. In addition, if desired, the injectable pharmaceutical compositions may contain minor

amounts of nontoxic auxiliary substances, such as wetting agents, pH buffering agents, and the like. Physiologically compatible buffers include, but are not limited to, Hanks's solution, Ringer's solution, or physiological saline buffer. If desired, absorption enhancing preparations (for example, liposomes), may be utilized.

[0059] For transmucosal administration, penetrants appropriate to the barrier to be permeated may be used in the formulation.

[0060] Pharmaceutical formulations for parenteral administration, *e.g.*, by bolus injection or continuous infusion, include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or other organic oils such as soybean, grapefruit or almond oils, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[0061] For oral administration, the compound(s) or combination of compounds disclosed herein can be formulated readily by combining the active compound with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compound or combination of compounds disclosed herein to be formulated as tablets, film coated tablets, pills, dragees, capsules, liquids, gels, get caps, pellets, beads, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by combining the active compound with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable

excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses. In addition, stabilizers can be added. All formulations for oral administration should be in dosages suitable for such administration. In some embodiments, formulations of the compound(s) or combination of compounds disclosed herein with an acceptable immediate release dissolution profile and a robust, scalable method of manufacture are disclosed.

[0062] Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

[0063] For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[0064] For administration by inhalation, the compound or combination of compounds disclosed herein is conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0065] Further disclosed herein are various pharmaceutical compositions well known in the pharmaceutical art for uses that include intraocular, intranasal, and intraauricular delivery. Suitable penetrants for these uses are generally known in the art. Pharmaceutical compositions for intraocular delivery include aqueous ophthalmic solutions of the active compounds in water-soluble form, such as eyedrops, or in gellan gum (Shedden et al., Clin. Ther., 23(3):440-50 (2001)) or hydrogels (Mayer et al., Ophthalmologica, 210(2):101-3 (1996)); ophthalmic ointments; ophthalmic suspensions, such as microparticulates, drug-containing small polymeric particles that are suspended in a liquid carrier medium (Joshi, A., J. Ocul. Pharmacol., 10(1):29-45 (1994)), lipid-soluble formulations (Alm et al., Prog. Clin. Biol. Res., 312:447-58 (1989)), and microspheres (Mordenti, Toxicol. Sci., 52(1):101-6 (1999)); and ocular inserts. All of the above-mentioned references are incorporated herein by reference in their entireties. Such suitable pharmaceutical formulations are most often and preferably formulated to be sterile, isotonic and buffered for stability and comfort. Pharmaceutical compositions for intranasal delviery may also include drops and sprays often prepared to simulate in many respects nasal secretions to ensure maintenance of normal ciliary action. As disclosed in Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, PA (1990), which is incorporated herein by reference in its entirety, and well-known to those skilled in the art, suitable formulations are most often and preferably isotonic, slightly buffered to maintain a pH of 5.5 to 6.5, and most often and preferably include antimicrobial preservatives and appropriate drug stabilizers. Pharmaceutical formulations for intraauricular delivery include

suspensions and ointments for topical application in the ear. Common solvents for such aural formulations include glycerin and water.

[0066] The compound(s) or combination of compounds disclosed herein may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

[0067] In addition to the formulations described previously, the compound or combination of compounds disclosed herein may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compound or combination of compounds disclosed herein may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0068] For hydrophobic compounds, a suitable pharmaceutical carrier may be a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. A common cosolvent system used is the VPD co-solvent system, which is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant Polysorbate 80™, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of POLYSORBATE 80™; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, *e.g.*, polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose.

[0069] Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by those

skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

[0070] Agents intended to be administered intracellularly may be administered using techniques well known to those of ordinary skill in the art. For example, such agents may be encapsulated into liposomes. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external micro-environment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. The liposome may be coated with a tissue-specific antibody. The liposomes will be targeted to and taken up selectively by the desired organ. Alternatively, small hydrophobic organic molecules may be directly administered intracellularly.

[0071] Additional therapeutic or diagnostic agents may be incorporated into the pharmaceutical compositions. Alternatively or additionally, pharmaceutical compositions may be combined with other compositions that contain other therapeutic or diagnostic agents.

Methods of Administration

[0072] The compound(s) or combination of compounds disclosed herein or pharmaceutical compositions may be administered to the patient by any suitable means. Non-limiting examples of methods of administration include, among others, (a) administration though oral pathways, which includes administration in capsule, tablet, granule, spray, syrup, or other such forms; (b) administration through non-oral pathways such as rectal, vaginal, intraurethral, intraocular, intranasal, or intraauricular, which includes administration as an aqueous suspension, an oily preparation or the like or as a drip, spray, suppository, salve, ointment or the like; (c) administration via injection, subcutaneously, intraperitoneally, intravenously, intramuscularly, intradermally, intraorbitally, intracapsularly, intraspinally, intrasternally, or the like, including infusion pump delivery; (d) administration locally such as by injection directly in the renal or cardiac area, e.g., by depot implantation; as well as (e) administration topically; as deemed appropriate by those of skill in the art for

bringing the compound or combination of compounds disclosed herein into contact with living tissue.

[0073] Pharmaceutical compositions suitable for administration include compositions where the compound(s) or combination of compounds disclosed herein is contained in an amount effective to achieve its intended purpose. The therapeutically effective amount of the compound or combination of compounds disclosed herein required as a dose will depend on the route of administration, the type of animal, including human, being treated, and the physical characteristics of the specific animal under consideration. The dose can be tailored to achieve a desired effect, but will depend on such factors as weight, diet, concurrent medication and other factors which those skilled in the medical arts will recognize. More specifically, a therapeutically effective amount means an amount of compound effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated. Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0074] As will be readily apparent to one skilled in the art, the useful *in vivo* dosage to be administered and the particular mode of administration will vary depending upon the age, weight and mammalian species treated, and the specific use for which the compound or combination of compounds disclosed herein are employed. The determination of effective dosage levels, that is the dosage levels necessary to achieve the desired result, can be accomplished by one skilled in the art using routine pharmacological methods. Typically, human clinical applications of products are commenced at lower dosage levels, with dosage level being increased until the desired effect is achieved. Alternatively, acceptable *in vitro* studies can be used to establish useful doses and routes of administration of the compositions identified by the present methods using established pharmacological methods.

[0075] As used herein, a "dosage" refers to the amount of the active pharmaceutical ingredients (e.g., minocycline and colistin).

[0076] In non-human animal studies, applications of potential products are commenced at higher dosage levels, with dosage being decreased until the desired effect is no longer achieved or adverse side effects disappear. The dosage may range broadly, depending

upon the desired effects and the therapeutic indication. Typically, dosages may be between about 0.1 mg/kg and 4000 mg/kg body weight, preferably between about 1 mg/kg and 1000 mg/kg body weight. Alternatively dosages may be based and calculated upon the surface area of the patient, as understood by those of skill in the art.

[0077] Depending on the severity and responsiveness of the condition to be treated, dosing can also be a single administration of a slow release composition, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved. The amount of a composition to be administered will, of course, be dependent on many factors including the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician. The compound or combination of compounds disclosed herein may be administered orally or via injection at a dose from 0.1 mg/kg to 4000 mg/kg of the patient's body weight per day. The dose range for adult humans is generally from 100 mg/day to 100 g/day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of the compound or combination of compounds disclosed herein which is effective at such dosage or as a multiple of the same, for instance, units containing 100 mg to 50 g (for example, from about 200 mg to 50 g, from about 400 mg to 20 g, from about 800 mg to 10 g, or from about 1 g to 5 g). The precise amount of compound administered to a patient will be the responsibility of the attendant physician. However, the dose employed will depend on a number of factors, including the age and sex of the patient, the precise disorder being treated, and its severity. Also, the route of administration may vary depending on the condition and its severity. A typical dose of minocycline is from 1 mg to 10 mg per kg of body weight, for example from 1.5 mg to 5 mg per kg of body weight, depending on such parameters. In some embodiments, a dosage of minocycline can be from about 1 mg to 1000 mg, for example, from 10 mg to 500 mg, from 50 mg to 400 mg, from 100 mg to 400 mg, or from 150 mg to 250 mg. A typical dose of colistin is from 1 mg to 20 mg per kg of body weight, for example from 1.5 mg to 15 mg per kg of body weight, depending on factors including the age and sex of the patient, the precise disorder being treated, and its severity. In some embodiments, a dosage of colistin can be from about 1 mg to 10000 mg, for example, from 10 mg to 5000 mg, from 100 mg to 3000 mg, from 200 mg to 2000 mg, or from 400 mg to 1000 mg. In

some embodiments, the minocycline and colistin can be administered in a weight ratio from 10:1 to 1:10, for example, from 5:1 to 1:5, from 4:1 to 1:4, from 3:1 to 1:3, from 2:1 to 1:2, or about 1:1. A physician will be able to determine the required dosage of minocycline and colistin for any particular subject.

[0078] The exact formulation, route of administration and dosage for the pharmaceutical compositions of the compound or combination of compounds disclosed herein can be chosen by the individual physician in view of the patient's condition. (*See, e.g.*, Fingl *et al.* 1975, in "The Pharmacological Basis of Therapeutics," which is hereby incorporated herein by reference, with particular reference to Ch. 1). Typically, the dose range of the composition administered to the patient can be from about 0.1 to about 4000 mg/kg of the patient's body weight. The dosage may be a single one or a series of two or more given in the course of one or more days, as is needed by the patient. In instances where human dosages for compounds have been established for at least some condition, the present disclosure will use those same dosages, or dosages that are between about 0.1% and about 5000%, more preferably between about 25% and about 1000% of the established human dosage. Where no human dosage is established, as will be the case for newly-discovered pharmaceutical compounds, a suitable human dosage can be inferred from ED_{50} or ID_{50} values, or other appropriate values derived from *in vitro* or *in vivo* studies, as qualified by toxicity studies and efficacy studies in animals.

[0079] It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity or organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administrated dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

[0080] Although the exact dosage will be determined on a drug-by-drug basis, in most cases, some generalizations regarding the dosage can be made. In cases of administration of a pharmaceutically acceptable salt, dosages may be calculated as the free base. In some embodiments, the composition is administered 1 to 4 times per day. Alternatively the compositions of the compound or combination of compounds disclosed herein may be administered by continuous intravenous infusion, preferably at a dose of each active ingredient up to 100 g per day. As will be understood by those of skill in the art, in certain situations it may be necessary to administer the compound disclosed herein in amounts that exceed, or even far exceed, the above-stated, preferred dosage range in order to effectively and aggressively treat particularly aggressive diseases or infections. In some embodiments, the compound or combination of compounds disclosed herein will be administered for a period of continuous therapy, for example for a week or more, or for months or years.

[0081] In some embodiments, the dosing regimen of the compound(s) or combination of compounds disclosed herein is administered for a period of time, which time period can be, for example, from at least about 1 day to at least about 3 days, from at least about 1 day to at least about 1 week, from at least about 1 day to at least about 10 days, from at least about 1 week to at least about 2 weeks, from at least about 1 week to at least about 4 weeks, from at least about 4 weeks to at least about 8 weeks, from at least about 4 weeks to at least about 12 weeks, from at least about 4 weeks to at least about 16 weeks, or longer. The dosing regimen of the compound(s) or combination of compounds disclosed herein can be administered three times a day, twice a day, daily, every other day, three times a week, every other week, three times per month, once monthly, substantially continuously or continuously.

[0082] In some embodiments described herein, the minocycline and the colistin may be administered to the subject simultaneously. In some embodiments, the minocycline and colistin may be administered to the subject sequentially. In some embodiments, the minocycline is administered to the subject prior to the administration of colistin to the subject. In some embodiments, the minocycline is administered to the subject subsequent to the administration of colistin to the subject.

[0083] In some embodiments, the minocycline and the colistin may be administered using the same route of administration. For example, in some embodiments, the minocycline and colistin may both be administered orally. In some embodiments, the minocycline and colistin may both be administered parenterally. In some embodiments, the minocycline and colistin may both be administered intravenously.

[0084] In some embodiments, the minocycline and colistin may be administered using different routes of administration.

[0085] Some embodiments provide a method to use an effective amount of the combination of minocycline and colistin disclosed herein in the treatment of bacterial infection in a subject comprising administering to the subject a dosage of the combination of minocycline and colistin disclosed herein containing an amount of about 0.1 g to about 100 g of drug per dose of the compound or combination of compounds disclosed herein, orally, three times per month, once monthly, once weekly, once every three days, once every two days, once per day, twice per day, or three times per day substantially continuously or continuously, for the desired duration of treatment.

[0086] Some embodiments provide a method to use an effective amount of the combination of minocycline and colistin disclosed herein in the treatment of a bacterial infection in a subject susceptible to acute kidney injury comprising administering to the subject a dosage of the minocycline and colistin containing an amount of from 0.1 mg to about 4000 mg of each of minocycline and colistin per kilogram of body weight per dose of minocycline and colistin, orally, three times per month, once monthly, once weekly, once every three days, once every two days, once per day, twice per day, or three times per day substantially continuously or continuously, for the desired duration of treatment.

[0087] Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the modulating effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

[0088] Dosage intervals can also be determined using MEC value. In some embodiments, compositions can be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, for example, between 15-30%, 20-45%, 25-50%, 30-55%, 35-60%, 40-65%, 45-70%, 50-75%, 55-80%, 60-90%, 65-75%, 70-80%, 75-85%, 15-90%, 20-90%, 25-90%, 30-90%, 35-90%, 40-90%, 45-90%, 50-90%, 55-90%, 60-90%, 65-90%, 70-90%, 75-90%, or 80-90%. In some embodiments, compositions can be administered using a regimen which maintains plasma levels above the MEC for 20-90% of the time. In some embodiments, compositions can be administered using a regimen which maintains plasma levels above the MEC for 30-90% of the time, between 40-90% and most typically between 50-90%.

[0089] In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

[0090] The amount of composition administered may be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

[0091] The compound(s) or combination of compounds disclosed herein can be evaluated for efficacy and toxicity using known methods. For example, the toxicology of the compound or combination of compounds disclosed herein may be established by determining *in vitro* toxicity towards a cell line, such as a mammalian, and preferably human, cell line. The results of such studies are often predictive of toxicity in animals, such as mammals, or more specifically, humans. Alternatively, the toxicity of the compound or combination of compounds disclosed herein in an animal model, such as mice, rats, rabbits, or monkeys, may be determined using known methods. The efficacy of the compound or combination of compounds disclosed herein may be established using several recognized methods, such as *in vitro* methods, animal models, or human clinical trials. Recognized *in vitro* models exist for nearly every class of condition, including but not limited to cancer, cardiovascular disease, and various immune dysfunction. Similarly, acceptable animal models may be used to establish efficacy of chemicals to treat such conditions. When selecting a model to determine efficacy, the skilled artisan can be guided by the state of the art to choose an appropriate

model, dose, and route of administration, and regime. Of course, human clinical trials can also be used to determine the efficacy of a compound in humans.

[0092] The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accompanied with a notice associated with the container in form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. Such notice, for example, may be the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product insert. Compositions comprising the compound or combination of compounds disclosed herein formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

[0093] An effective amount of the minocycline and colistin disclosed herein may be determined by one of ordinary skill in the art. It will be understood that the specific dose level and frequency of dosage for any particular subject may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the species, age, body weight, general health, sex and diet of the subject, the mode and time of administration, rate of excretion, drug combination, and severity of the particular condition. Preferred subjects for treatment include animals, most preferably mammalian species such as humans, and domestic animals such as dogs, cats and the like, subject to portal hypertension.

[0094] Pharmaceutical compositions comprising the minocycline and colistin disclosed herein capable of treating bacterial infection in an amount effective therefore, and a pharmaceutically acceptable vehicle or diluent are also disclosed. The compositions of the present disclosure may contain other therapeutic agents as described below, and may be formulated, for example, by employing conventional solid or liquid vehicles or diluents, as well as pharmaceutical additives of a type appropriate to the mode of desired administration (for example, excipients, binders, preservatives, stabilizers, flavors, etc.) according to

techniques such as those well known in the art of pharmaceutical formulation or called for by accepted pharmaceutical practice.

[0095] The minocycline and colistin dosages disclosed herein may be administered by any suitable means, for example, orally, such as in the form of tablets, capsules, granules or powders; sublingually; buccally; parenterally, such as by subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion techniques (e.g., as sterile injectable aqueous or non-aqueous solutions or suspensions); nasally such as by inhalation spray; topically, such as in the form of a cream or ointment; or rectally such as in the form of suppositories; in dosage unit formulations containing non-toxic, pharmaceutically acceptable vehicles or diluents.

[0096] The minocycline and colistin disclosed herein, for example, may be administered in a form suitable for immediate release or extended release. Immediate release or extended release may be achieved by the use of suitable pharmaceutical compositions comprising minocycline and colistin, or, particularly in the case of extended release, by the use of devices such as subcutaneous implants or osmotic pumps.

[0097] The minocycline and colistin disclosed herein may also be administered liposomally. For example, the active substance can be utilized in a composition such as tablet, capsule, solution or suspension minocycline and colistin disclosed herein or in topical form for wound healing (0.01 to 5% by minocycline and colistin disclosed herein, 1 to 5 treatments per day).

[0098] The minocycline and colistin disclosed herein may be compounded in a conventional manner with a physiologically acceptable vehicle or carrier, excipient, binder, preservative, stabilizer, flavor, etc., or with a topical carrier.

[0099] The minocycline and colistin disclosed herein can also be formulated in compositions such as sterile solutions or suspensions for parenteral administration. The minocycline and colistin disclosed herein may be compounded with a physiologically acceptable vehicle, carrier, excipient, binder, preservative, stabilizer, etc., in a unit dosage form as called for by accepted pharmaceutical practice. The amount of active substance in these compositions or preparations is preferably such that a suitable dosage in the range indicated is obtained.

[0100] Exemplary compositions for oral administration include suspensions which may contain, for example, microcrystalline cellulose for imparting bulk, alginic acid or sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and sweeteners or flavoring agents such as those known in the art; and immediate release tablets which may contain, for example, microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and/or lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants such as those known in the art. Molded tablets, compressed tablets or freeze-dried tablets are exemplary forms which may be used. Exemplary compositions include those formulating the compound or combination of compounds disclosed herein with fast dissolving diluents such as mannitol, lactose, sucrose and/or cyclodextrins. Also included in such formulations may be high molecular weight excipients such as celluloses (avicel) or polyethylene glycols (PEG). Such formulations may also include an excipient to aid mucosal adhesion such as hydroxy propyl cellulose (HPC), hydroxy propyl methyl cellulose (HPMC), sodium carboxy methyl cellulose (SCMC), maleic anhydride copolymer (e.g., Gantrez), and agents to control release such as polyacrylic copolymer (e.g., Carbopol 934). Lubricants, glidants, flavors, coloring agents and stabilizers may also be added for ease of fabrication and use.

[0101] Exemplary compositions for nasal aerosol or inhalation administration include solutions in saline which may contain, for example, benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other solubilizing or dispersing agents such as those known in the art.

[0102] Exemplary compositions for parenteral administration include injectable solutions or suspensions which may contain, for example, suitable non-toxic, parenterally acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution, an isotonic sodium chloride solution, or other suitable dispersing or wetting and suspending agents, including synthetic mono- or diglycerides, and fatty acids, including oleic acid.

[0103] Exemplary compositions for rectal administration include suppositories which may contain, for example, a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquify and/or dissolve in the rectal cavity to release the drug.

[0104] Exemplary compositions for topical administration include a topical carrier such as Plastibase (mineral oil gelled with polyethylene). For example, the compound or combination of compounds disclosed herein may be administered topically to treat peripheral vascular diseases and as such may be formulated as a cream or ointment.

[0105] In some embodiments, the composition disclosed herein can comprise at least 0.1% (w/w), 0.2% (w/w), 0.3% (w/w), 0.4% (w/w), 0.5% (w/w), 0.6% (w/w), 0.7% (w/w), 0.8% (w/w), 0.9% (w/w), 1.0% (w/w), 1.1% (w/w), or 1.2% (w/w) of a preservative. In some embodiments, the topical composition disclosed herein can comprise 0.1% (w/w), 0.2% (w/w), 0.3% (w/w), 0.4% (w/w), 0.5% (w/w), 0.6% (w/w), 0.7% (w/w), 0.8% (w/w), 0.9% (w/w), 1.0% (w/w), 1.1% (w/w), 1.2% (w/w), 1.5% (w/w), 2% (w/w), 3% (w/w), 4% (w/w), 5% (w/w), 6% (w/w), 7% (w/w), 8% (w/w), 9% (w/w), 10% (w/w), 20% (w/w) or 30% (w/w) of a preservative or a range defined by any two of the preceding values. In some embodiments, the preservative can include one or more components, two or more components or three or more components.

[0106] In some embodiments, the composition disclosed herein can comprise at least 0.1% (w/w), 0.2% (w/w), 0.3% (w/w), 0.4% (w/w), 0.5% (w/w), 0.6% (w/w), 0.7% (w/w), 0.8% (w/w), 0.9% (w/w), 1.0% (w/w), 1.1% (w/w), or 1.2% (w/w) of a preservative including phenoxyethanol, propyl paraben, and methyl paraben. In some embodiments, the topical composition disclosed herein can comprise 0.1% (w/w), 0.2% (w/w), 0.3% (w/w), 0.4% (w/w), 0.5% (w/w), 0.6% (w/w), 0.7% (w/w), 0.8% (w/w), 0.9% (w/w), 1.0% (w/w), 1.1% (w/w), 1.2% (w/w), 1.5% (w/w), 2% (w/w), 3% (w/w), 4% (w/w), 5% (w/w), 6% (w/w), 7% (w/w), 8% (w/w), 9% (w/w), 10% (w/w), 20% (w/w) or 30% (w/w) of a preservative including phenoxyethanol, propyl paraben, and methyl paraben or a range defined by any two of the preceding values.

[0107] In some embodiments, the composition may include colorants, deodorants, fragrances, perfumes, anti-foaming agents, lubricants, natural moisturizing agents, skin conditioning agents, skin protectants, skin benefit agents, solvents, solubilizing agents, suspending agents, wetting agents, humectants, propellants, dyes, pigments, and combinations thereof.

[0108] In some embodiments, the composition may include additional components added to enhance the odor, texture or color of the composition. For example, fragrances may be added to enhance odor. For example, emulsifiers or inert spheres may be added to enhance texture. For example, colorants may be added to enhance color.

[0109] In some embodiments, the composition may be applied to a body portion, such as a hand, foot, knee, elbow, and the like to treat pain and/or inflammation of the body portion. The composition may be applied by any suitable means, such as rubbing, spraying, rolling, wiping, and the like, and massaged into the body portion to be treated.

[0110] In some embodiments, the minocycline and colistin as disclosed and described herein and/or topical compositions thereof can be used in combination therapy with at least one other agent. In some embodiments, the minocycline and colistin disclosed herein and/or topical composition thereof is administered concurrently with the administration of another agent, which may be part of the same topical composition as the compound of the present invention or a different composition. In other embodiments, a topical composition of the present invention is administered prior or subsequent to administration of another agent.

[0111] In some embodiments the compositions described herein are incorporated into a patch or film for transdermal drug delivery. In some embodiments, such patches further comprise a porous or resorbable film, an active pharmaceutical agent, and optionally a transdermal carrier or penetration enhancer. Exemplary transdermal carriers include dimethylsulfoxide;1-dodecylazacycloheptan-2-one or laurocapran; dimethylacetamide; dimethylformamide; lauric acid; myristic acid; capric acid; caprylic acid; oleic acid; diethylene glycol; tetraethylene glycol; terpenes; essential oils of eucalyptus, chenopodium and ylang-ylang; dimethyl isosorbide; Oxazolidinones such as 4-decyloxazolidin-2-one; 2-pyrrolidone; N-methyl-2-pyrrolidone; urea; EDTA; Sodium Glycolate; polysorbates; sodium deoxycholate; polyethylene glycol;PLA/PLGA nanoparticles; polymer nanoparticles; block-copolymer nanoparticles, especially those comprising Pluronic®-type polyethylene oxide-block-polypropylene oxide copolymers; porous silica nanoparticles; metallic nanoparticles, especially those comprising gold, palladium, and iron; metal oxide nanoparticles, especially those comprising TiO_2 and Al_2O_3; short chain alcohols such as ethanol, propanol, and butanol; and oils such as mineral oil and coconut oil. In some embodiments the compositions

described herein are incorporated into an adhesive for a transdermal patch. In some further embodiments, the compositions described herein are incorporated into a resorbable film. In some embodiments, the active pharmaceutical agent is contained within a separate reservoir layer. In some embodiments, the transdermal patch consists of a single layer. In some embodiments, the transdermal patch is constructed in multiple layers.

Kits

[0112] Some embodiments of the present invention include kits comprising minocycline, colistin or combination thereof. Some kits include a single use container comprising minocycline, colistin or combination thereof. Single use containers include ampules, vials, and the like. The single-use container can comprise a lyophilized formulation of minocycline, colistin or combination thereof. Some kits include a diluent for reconstituting the lyophilized formulations of minocycline, colistin or combination thereof.

[0113] In some embodiments, minocycline, colistin, or combination thereof may be prepared for single-dosage use. In this embodiment, the solutions of the invention are lyophilized in individual vials such as 20-mL vials. Upon lyophilization, the vials are stoppered with any acceptable stopper. The stoppered vials are then shipped for use. When needed, the vials can be reconstituted by adding sufficient diluents to achieve the desired concentration of minocycline and/or colistin. The concentration of reconstituted solutions may be easily determined by those of ordinary skill in the art. Any pharmaceutically acceptable diluent may be used. Examples of such diluents include but are not limited to water, 0.9% saline, Lactated Ringer's injection solution and dextrose solutions including 5% dextrose (5DW).

[0114] In some embodiments, the diluent does not comprise a pharmaceutically acceptable oil (e.g., polyoxyethylene hydrogenated castor oils), a pyridine-containing compound (e.g., nicotinamide), gluconate, an antioxidant, an alcohol (e.g., a polyhydric alcohol, such as, propylene glycol, ethylene glycol), glycerol, polyethylene glycol, a pyrrolidone-containing compound, a water-miscible local anaesthetic (e.g., procaine, tetracaine), urea, lactose, or a dehydrating agent (e.g., ethyl acetate, acetic anhydride, absolute

ethanol, ethyl acetate, acetic anhydride, and mixtures thereof). In some embodiments, the diluent does not comprise a tetracycline-solubilizing cosolvent.

[0115] In some embodiments, the diluent contains the divalent or trivalent cation. For example, some embodiments include kits that comprise a first container comprising a diluent that comprises an aqueous solution of a divalent or trivalent cation; and a second container comprising a solid composition soluble in the diluent, wherein the solid composition comprises minocycline in an amount such that the molar ratio of the divalent or trivalent cation to minocycline is greater than about 2:1. In some embodiments, the diluent comprises an acid, e.g., HCl. In some embodiments, the diluent comprises a buffer. In some embodiments, the buffer is sodium acetate.

[0116] More embodiments include kits comprising a first container comprising a diluent that comprises an aqueous solution of a divalent or trivalent cation; and a second container comprising a solid composition soluble in the diluent, wherein the solid composition comprises a minocycline, colistin, or a combination thereof in an amount such that the molar ratio of the divalent or trivalent cation to tetracycline antibiotic is greater than 3:1.

[0117] More embodiments include single use vials comprising any composition wherein the vial comprises an amount of minocycline, colistin or combination thereof of at least 100 μg, 200 μg, 300 μg, 400 μg, 500 μg, 600 μg, 700 μg, 800 μg, 900 μg, 1000 μg. In some embodiments, the vial comprises an amount of minocycline, colistin or combination thereof of at least 1 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 105 mg, 110 mg, 115 mg, 120 mg, 125 mg, and 130 mg. In some embodiments, the vial comprises an amount of minocycline, colistin or combination thereof of at least 100 mg, 200 mg, 300 mg, 400 mg, and 500 mg. In some embodiments, the vial comprises about 1000 mg of minocycline, colistin or combination thereof.

Examples

[0118] Embodiments of the present application are disclosed in further detail in the following example, which is not in any way intended to limit the scope of the present disclosure.

Example 1

[0119] In this example, an analysis was conducted on the co-administration of colistin with minocycline to determine if the co-administration reduces nephrotoxicity in subjects as compared to administration of colistin alone. The patients included in the study were at least 18 years old and hospitalized with hospital acquired pneumonia (HAP), ventilator-associated pneumonia (VAP) and/or sepsis. The patents received either intravenous colistin alone or co-administration of intravenous minocycline and intravenous colistin. Each patient received at least 3 days of intravenous colistin, and the overlap of co-administration was at least three days. Table 1 presents the study drug administration by treatment group.

Table 1: Study drug administration by treatment group

	colistin (N=5,025)	**co-administration of colistin and minocycline (N=95)**	**p-value**
# of Days on treatment:			
# of days on minocycline		10.55±8.34	n/a
# of days on colistin	9.99±8.48	13.23 ± 11.53	0.003
# of overlap days for "co-administration"		7.36 ± 4.39	n/a
Days from 1st dose to last dose (including skipped days)			
minocycline	n/a	17.91±31.39	n/a
colistin	13.34±20.92	22.68±36.67	<0.001
Days from admission to initiation of study drug	14.41±19.83	14.36±23.08	0.091
For Co-administration cohort, (%, n)			
minocycline was initiated		18.9% (n=18)	

prior to colistin			
minocycline was initiated after colistin		45.3% (n=43)	
minocycline and colistin was initiated at same time		35.8% (n=34)	

[0120] The outcomes based on treatment groups are provided in Table 2. The data shows that the rate of acute kidney injury in subjects treated with a co-administration of colistin and minocycline was significantly lower than the rate of acute kidney injury in subjects treated with colistin alone.

Table 2: Outcomes of Subjects by Treatment Groups

Outcomes	**colistin alone (N=5,025)**	**colistin + minocycline (N=95)**	**p-value**
Acute Kidney Injury rate, (%)	23.0%	11.6%	0.009
Mortality, (%)	29.9%	31.6%	0.715
30-day all-cause re-admission rate, (%)	27.0%	30.8%	0.492
60-day all-cause re-admission rate, (%)	35.6%	46.2%	0.080

Example 2

[0121] In this example, an analysis was conducted on the co-administration of colistin with minocycline to determine if the co-administration reduces the incidence of acute renal failure in patients admitted to an intensive care unit. A retrospective cohort study was conducted using the Premier Research database. Patients were included for analysis if they met the following criteria: (1) age ≥18 years; (2) admitted to an intensive care unit (ICU) during study drug initiation; (3) had a primary ICD-9 diagnosis of pneumonia or sepsis; (4) received at least 3 days of colistin (COL) therapy; and (5) received at least 3 days of overlap if co-administrated with minocycline (MIN) or tigecycline (TIG). The frequency of acute renal failure, defined by ICD-9 code 584.XX or ICD-10 code N17.XX, was compared between patients who received COL without MIN or TIG (COL), COL with MIN (COL-MIN), or COL with TIG (COL-TIG). Patients who received COL with both MIN and TIG

were classified as COL-MIN. Patients in any cohort may have received additional antibiotics at any time during their hospitalization.

[0122] A total of 4,602 patients received colistin and met the inclusion criteria; 95 patients received COL-MIN and 995 received COL-TIG. The occurrence of acute renal failure was lowest among patients receiving COL-MIN. The occurrence of acute renal failure was similar between patients receiving COL and COL-TIG. The data provided in Table 3 shows that co-administration of minocycline with colistin in ICU patients may reduce the occurrence of colistin-associated acute renal failure. Similar reduction was not seen with colistin in combination with tigecycline.

Table 3: Incidence of Acute Renal Failure by Treatment Group

	COL	**COL-TIG**	**COL-MIN**
Unadjusted outcomes	n=3,512	n=995	N=95
Acute Renal Failure	21.2%	25.6%	11.6%

Example 3

[0123] In this example, an analysis was conducted on the co-administration of colistin with minocycline to determine if the co-administration reduces the incidence of acute renal failure in subjects as compared to administration of colistin alone. Patients were included for analysis if they met the following criteria: (1) age ≥18 years; (2) admitted to an intensive care unit (ICU) during study drug initiation; snd (3) had a primary ICD-9 diagnosis of pneumonia or sepsis. ICD-9 code 584.XX or ICD-10 code N1 was used to define acute renal failure. Baseline comparisons, 1:8 propensity score matching (PSM), and conformatory logistic regression analyses were conducted. PSM was conducted using nearest neighbor matching with exact matches on baseline renal disease and region. Regression variables included age, gender, race, diagnosis, use of meropenem or tigecycline, discharge year, hospital size, region, payor type, 17 Charlson comorbidities, other medications associated with acute renal failure, length of stay prior to initiation of study drugs, and mechanical ventilation use.

[0124] A total of 5,025 patients received colistin alone (COL) and met the inclusion criteria; 95 patients received minocycline in combination with colistin (COL-MIN). In PSM analysis, 86 (90.53%) of COL-MIN co-administration patients were matched 1:8 with 688 patients receiving colistin without minocycline. Without risk factor adjustment, COL-MIN patients were significantly less likely to experience acute renal failure compared to COL patients (11.6% vs. 23.0%). In-hospital mortality and 30-day all-cause re-admission were similar between groups (Table 4). Acute renal failure rates were consistently and numerically lower in COL-MIN patients regardless the length of colistin duration; similar findings were observed in those patients with (10.2% vs. 20.6%) and without (13.0% vs. 24.6%) baseline chronic renal disease (Table 5).

[0125] PSM results were consistent with the unadjusted analysis: patients receiving COL-MIN were less likely to experience acute renal failure compared to those receiving COL (11.6% vs. 24.7%, OR 0.401, p=0.007). Confirmatory logistic regression found an odds ratio of 0.390 for ARF in COL-MIN vs. COL patients (p=0.005). Both PSM method and conventional logistic regression modeling technique confirmed that mortality and 30-day readmission rates remained similar between groups (Table 4). Accordingly, co-administration of minocycline with colistin in ICU patients may reduce the occurrence of colistin-associated acute renal failure.

Table 4: Unadjusted and adjusted outcomes in patients treated with COL or COL-MIN

	COL	**COL-MIN**	**Odds Ratio (OR)**	**p-value**
Unadjusted outcomes	n=5025	n=95		
Acute Renal Failure	23.0%	11.6%	0.438	0.009
Mortality	29.9%	31.6%	1.085	0.715
30-day readmission	23.0%	11.6%	0.438	0.009
PSM (1:8 matching)	n=688	n=86		
Acute Renal Failure	24.7%	24.7%	0.401	0.007
Mortality	32.0%	32.0%	1.027	0.913
30-day readmission	28.0%	28.0%	1.067	0.833
Logistic regression model	n=5025	n=95		
Acute Renal Failure			0.390	0.005
Mortality			0.913	0.699
30-day readmission			1.112	0.702

Table 5: Effect of number of days on COL/COL-MIN and chronic renal disease status on unadjusted incidence of acute renal failure

		Unadjusted Acute Renal Failure Rate		
Risk Factor	**Category**	**COL**	**COL-MIN**	**p-value**
Duration of colistin treatment	3-5 days	305/1596 (19.1%)	2/21 (9.5%)	0.401
	6-8 days	268/1223 (21.9%)	2/19 (10.5%)	0.398
	9-13 days	283/1145 (24.7%)	3/27 (11.1%)	0.117
	≥ 14 days	300/1061 (28.3%)	4/28 (14.3%)	0.135
Chronic renal disease status	Yes	410/1987 (20.6%)	5/49 (10.2%)	0.073
	No	746/3038 (24.6%)	6/46 (13.0%)	0.071

[0126] Although the present disclosure has been described with reference to embodiments and examples, it should be understood that numerous and various modifications can be made without departing from the spirit of the present disclosure. Accordingly, the present disclosure is limited only by the following claims.

[0127] All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited herein, to the extent that they are not already, are hereby incorporated by reference in their entirety. In the event that one or more of the incorporated literature and similar materials differ from or contradict this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

WHAT IS CLAIMED IS:

1. A method of treating bacterial infection in a subject, comprising administering to the subject minocycline in combination with colistin.

2. The method of Claim 1, wherein the subject is susceptible to acute kidney injury.

3. The method of Claim 1 or 2, wherein the subject is suffering from a disease or disorder selected from the group consisting of myocardial infarction, congestive heart failure, peripheral vascular disease, coronary artery disease, chronic pulmonary disease, diabetes, dementia, peptic ulcer disease, renal disease, cancer, liver disease, paraplegia, hemiplegia, HIV/AIDS, and rheumatic disease.

4. The method of any one of Claims 1 to 3, wherein administering to the subject minocycline in combination with colistin reduces the risk of acute kidney injury.

5. The method of any one of Claims 1 to 4, wherein the bacterial infection is selected from the group consisting of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Chlamydia trachomatis*, *Mycoplasma pneumoniae*, and *Legionella pneumophila*, *Acinetobacter baumannii*, *Bartonella bacilliformis*, *Brucella species*, *Calymmatobacterium granulomatis*, *Campylobacter fetus*, *Francisella tularensis*, *Haemophilus ducreyi*, *Vibrio cholerae*, and *Yersinia pestis*.

6. The method of any one of Claims 1 to 5, wherein the subject has a glomerular filtration rate below about 90 mL/min/1.73 m^2.

7. The method of Claim 6, wherein the subject has a glomerular filtration rate between about 60 to about 90 mL/min/1.73 m^2.

8. The method of Claim 6, wherein the subject has a glomerular filtration rate between about 45 to about 60 mL/min/1.73 m^2.

9. The method of Claim 6, wherein the subject has a glomerular filtration rate between about 30 to about 45 mL/min/1.73 m^2.

10. The method of Claim 6, wherein the subject has a glomerular filtration rate between about 15 to about 30 mL/min/1.73 m^2.

11. The method of Claim 6, wherein the subject has a glomerular filtration rate below about 15 mL/min/1.73 m^2.

12. The method of any one of Claims 1 to 11, wherein the subject has a urine albumin level from about 30 to about 150 mg per day.

13. The method of any one of claims 1 to 11, wherein the subject has a urine albumin level from about 150 to about 300 mg per day.

14. The method of any one of claims 1 to 11, wherein the subject has a urine albumin level greater than about 300 mg per day.

15. The method of any one of claims 1 to 14, wherein the subject has a serum creatinine level between about 1 to about 5 mg/dL.

16. The method of claim 15, wherein the subject has a serum creatinine level between about 2 to about 5 mg/dL.

17. The method of claim 15, wherein the subject has a serum creatinine level between about 3 to about 5 mg/dL.

18. The method of any one of claims 1 to 17, wherein the subject has a creatinine clearance rate below about 90 mL/min.

19. The method of claim 18, wherein the subject has a creatinine clearance rate below about 60 mL/min.

20. The method of any one of claims 1 to 19, wherein the subject's blood urea nitrogen level between about 5 and about 20 mg/dL.

21. The method of any one of claims 1 to 19, wherein the subject's blood urea nitrogen level between about 20 and about 40 mg/dL.

22. The method of any one of claims 1 to 19, wherein the subject's blood urea nitrogen level between about 40 and about 60 mg/dL.

23. The method of any one of Claims 1 to 22, wherein the administration is oral, intravenous, intraperitoneal, intragastric, or intravascular administration.

24. The method of Claim 23, wherein the administration is intravenous administration.

25. The method of Claim 23, wherein said administration is oral administration.

26. The method of any one of Claims 1 to 26, wherein the dose of the minocycline administered is between 1 mg and 400 mg daily.

27. The method of Claim 26, wherein the dose of minocycline is 50 mg to 300 mg daily.

28. The method of Claim 26, wherein the dose of minocycline is 150 mg to 250 mg daily.

29. The method of any one of Claims 1 to 28, wherein the dose of the colistin administered is between 1 mg and 500 mg daily.

30. The method of Claim 29, wherein the dose of the colistin administered is between 100 mg and 400 mg daily.

31. The method of Claim 29, wherein the dose of the colistin administered is between 150 mg and 250 mg daily.

32. The method of any of Claims 1 to 31, wherein the minocycline is administered from one to four times daily.

33. The method of Claim 32 wherein the minocycline is administered once daily.

34. The method of Claim 32 wherein the minocycline is administered twice daily.

35. The method of any of Claims 1 to 34, wherein the minocycline is administered from one to four times daily.

36. The method of Claim 35 wherein the colistin is administered once daily.

37. The method of Claim 35 wherein the colistin is administered twice daily.

38. The method of any one of claims 1 to 37, wherein the minocycline and colistin are administered simultaneously.

39. The method of any one of claims 1 to 37, wherein the minocycline and colistin are administered sequentially.

40. A pharmaceutical composition comprising minocycline and colistin, and optionally comprising one or more pharmaceutically acceptable excipients.

41. The composition of claim 40, wherein the composition is formulated for oral, intravenous, intraperitoneal, intragastric, or intravascular administration.

42. A method of treating bacterial infection in a subject, comprising the steps of:

(a) administering a first administration of minocycline, colistin, or a combination thereof to the subject;

(b) obtaining a biological sample from the subject after said first administration;

(c) measuring the level of a kidney function parameter; and

(d) administering a suitable second administration of minocycline, colistin, or a combination thereof to the subject.

43. The method of Claim 42, wherein the subject is susceptible to acute kidney injury.

44. The method of Claim 43 or 44, wherein the subject is suffering from a disease or disorder selected from the group consisting of myocardial infarction, congestive heart failure, peripheral vascular disease, coronary artery disease, chronic pulmonary disease, diabetes, dementia, peptic ulcer disease, renal disease, cancer, liver disease, paraplegia, hemiplegia, HIV/AIDS, and rheumatic disease.

45. The method of any one of Claims 42 to 44, wherein administering to the subject minocycline in combination with colistin reduces the risk of acute kidney injury.

46. The method of any one of Claims 42 to 45, wherein the bacterial infection is selected from the group consisting of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Chlamydia trachomatis*, *Mycoplasma pneumoniae*, and *Legionella pneumophila*, *Acinetobacter baumannii*, *Bartonella bacilliformis*, *Brucella species*, *Calymmatobacterium granulomatis*, *Campylobacter fetus*, *Francisella tularensis*, *Haemophilus ducreyi*, *Vibrio cholerae*, and *Yersinia pestis*.

47. The method of any one of Claims 42 to 45, wherein the kidney function parameter is selected from the group consisting of glomerular filtration rate, urine albumin level, a serum creatinine level, creatine clearance rate, and blood urea nitrogen level.

48. The method of any one of Claims 1 to 47, wherein the first administration is oral, intravenous, intraperitoneal, intragastric, or intravascular administration.

49. The method of Claim 48, wherein said first administration is intravenous administration.

50. The method of Claim 48, wherein said first administration is oral administration.

51. The method of any one of Claims 1 to 50, wherein the second administration is oral, intravenous, intraperitoneal, intragastric, or intravascular administration.

52. The method of Claim 50, wherein said second administration is intravenous administration.

53. The method of Claim 50, wherein said second administration is oral administration.