TITLE OF THE INVENTION

BACTERIALCIDAL METHODS AND COMPOSITONS

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[0001] This work was supported by a National Institutes of Health Grant No. R01AI132638. The government may have certain rights to the invention.

RELATED APPLICATIONS/PATENTS

[0002] This application claims priority to U.S. Application Ser. No. 62/799,328, filed on January 31, 2019 as Attorney Docket No. 701586-094400PL01, the contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0003] Antibiotic resistance kills an estimated 700,000 people each year worldwide, and studies predict that this number could rise to 10 million by 2050, if efforts are not made to curtail resistance (Willyard, C. J. N. N. The drug-resistant bacteria that pose the greatest health threats. 543, 15 (2017)). Yet, the pace of resistance acquisition from mutation in pathogens is faster than clinical introduction of new antibiotics. There is an urgent need to develop unconventional ways to combat the resistance.

SUMMARY ~~OF THE INVENTION~~

[0004] The lethal effect of certain antibiotics occurs through the generation of Reactive Oxygen Species (ROS). Catalase, the ubiquitous key defense enzyme existing in most of the aerobic pathogens, is utilized to scavenge hydrogen peroxide, thus preventing downstream oxidative damage. It has now been shown that catalase can be optimally photoinactivated by blue light having a wavelength of about 400nm to about 430nm, and specifically, a wavelength of about 410 nm. Photoinactivation of catalase renders broad-SPECTRUM catalase-positive microbial pathogens highly susceptible to ROS-generating antimicrobials and/or immune cell attack. It has now been further determined that the antimicrobial effect of

photoinactivation is significantly and unexpectedly increased upon administration of a low-concentration of H_2O_2 and/or a ROS-generating agent.

[0005] In one aspect, the present disclosure refers to the use of a composition comprising a reactive oxygen species (ROS) in the manufacture of a medicament for treating a tissue of a subject infected with a catalase-positive microbe, wherein light is to be applied to the tissue of the subject infected with the catalase-positive microbe at a wavelength of 400nm to 430nm, wherein the catalase is inactivated, and the tissue is subsequently to be contacted with a composition comprising a reactive oxygen species (ROS). In one example, there is disclosed ~~the invention provides~~ a method of treating a tissue of a subject infected with a catalase-positive microbe, said method comprising the steps of: applying light to the tissue of the subject infected with the catalase-positive microbe at a wavelength of about 400nm to about 430nm, wherein the catalase is inactivated, and contacting the tissue with a composition comprising a diluted peroxide solution, thereby treating the tissue of the subject infected with the catalase-positive microbe.

[0006] In one ~~embodiment~~example, the wavelength is about 410nm.

[0007] In another example~~embodiment~~, the dose of the light is about 5 J/cm^2 to about 200 J/cm^2.

[0008] In yet another example~~embodiment~~, the dose of the light is about 15 J/cm^2.

[0009] In yet another example~~embodiment~~, the catalase-positive microbe is a fungal or bacterial microbe and the light is provided by a pulsed nanosecond laser.

[0010] In yet another example~~embodiment~~, the catalase-positive microbe is a fungal or bacterial microbe and the light is provided by a continuous wave LED.

[0011] In yet another example~~embodiment~~, the diluted peroxide solution is a hydrogen peroxide solution.

[0012] In yet another example~~embodiment~~, the hydrogen peroxide solution is between about 0.03% and about 0.3% hydrogen peroxide.

[0013] In yet another example~~embodiment~~, the method further comprises administering a ROS generating agent to the infected tissue of the subject.

[0014] In yet another example~~embodiment~~, the ROS generating agent is tobramycin, silver cation, iodine tincture, a gold nanoparticle, methylene blue, a β-lactam antibiotic, an aminoglycoside, a fluoroquinolone, an azole, a membrane-targeting polyene antifungal or a cell-wall targeting antifungal.

[0015] In yet another example~~embodiment~~, the tissue is skin, scalp or nails.

[0016] In yet another example~~embodiment~~, the catalase-positive microbe is eradicated.

[0017] In another aspect, the present disclosure refers to ~~the invention provides~~ a method of disinfecting an inanimate surface contaminated with a catalase-positive microbe, said method comprising the steps of: applying light to the inanimate surface at a wavelength of ~~about~~ 400nm to ~~about~~ 430nm,

wherein the catalase is inactivated, and subsequently contacting the inanimate surface with a composition comprising a diluted peroxide solution, thereby disinfecting the inanimate surface.

[0018] In one example~~embodiment~~, the wavelength is about 410nm.

[0019] In another example~~embodiment~~, the dose of the light is about 5 J/cm^2 to about 200 J/cm^2.

[0020] In yet another example~~embodiment~~, the dose of the light is about 15 J/cm^2.

[0021] In yet another example~~embodiment~~, the catalase-positive microbe is a fungal or bacterial microbe and the light is provided by a pulsed nanosecond laser.

[0022] In yet another example~~embodiment~~, the catalase-positive microbe is a fungal or bacterial microbe and the light is provided by a continuous wave LED.

[0023] In yet another example~~embodiment~~, the diluted peroxide solution is a hydrogen peroxide solution.

[0024] In yet another example~~embodiment~~, the hydrogen peroxide solution is between about 0.03% and about 0.3% hydrogen peroxide.

[0025] In yet another example~~embodiment~~, the method further comprises administering a ROS generating agent to the infected tissue of the subject.

[0026] In yet another example~~embodiment~~, the ROS generating agent is tobramycin, silver cation, iodine tincture, a gold nanoparticle, methylene blue, a β-lactam antibiotic, an aminoglycoside, a fluoroquinolone, an azole, a membrane-targeting polyene antifungal or a cell-wall targeting antifungal.

[0027] In yet another example~~embodiment~~, the inanimate surface is a material comprising metal, plastic, fabric, rubber, stone, composite surfaces or wood.

[0028] In yet another example~~embodiment~~, the catalase-positive microbe is eradicated.

[0029] In yet another aspect, the present disclosure refers to the use of a composition comprising a ROS generating agent in the manufacture of a medicament for treating a tissue of a subject infected with a catalase-positive microbe, wherein light from a pulsed nanosecond laser is to be applied to the tissue of the subject infected with the catalase-positive microbe at a wavelength of 400nm to 460nm, wherein the catalase is inactivated, and the tissue is subsequently to be contacted with a composition comprising a ROS generating agent. In one example, there is described ~~the invention provides~~ a method of treating a tissue of a subject infected with a catalase-positive microbe, said method comprising the steps of: applying light from a pulsed nanosecond laser to the tissue of the subject infected with the catalase-positive microbe at a wavelength of about 400nm to about 460nm, wherein the catalase is inactivated, and contacting the tissue with a composition comprising a ROS generating agent, thereby treating the tissue of the subject infected with the catalase-positive microbe.

[0030] In one ~~embodiment~~example, the wavelength is about 410nm.

[0031] In another example~~embodiment~~, the dose of the light is about 5 J/cm^2 to about 200 J/cm^2.

[0032] In yet another example~~embodiment~~, the dose of the light is about 15 J/cm^2.

[0033] In yet another example~~embodiment~~, the catalase-positive microbe is a fungal or bacterial microbe.

[0034] In yet another example~~embodiment~~, the diluted peroxide solution is a hydrogen peroxide solution.

[0035] In yet another example~~embodiment~~, the hydrogen peroxide solution is between about 0.03% and about 0.3% hydrogen peroxide.

[0036] In yet another example~~embodiment~~, the ROS generating agent is tobramycin, silver cation, iodine tincture, a gold nanoparticle, methylene blue, a β-lactam antibiotic, an aminoglycoside, a fluoroquinolone, an azole, a membrane-targeting polyene antifungal or a cell-wall targeting antifungal.

[0037] In yet another example~~embodiment~~, the tissue is skin, scalp or nails.

[0038] In yet another example~~embodiment~~, the catalase-positive microbe is eradicated.

[0039] In yet another aspect, the present disclosure refers to the use of a composition comprising a peroxide solution in the manufacture of a medicament for producing a synergistic antimicrobial effect in a tissue of a subject infected with a catalase-positive microbe, wherein light is to be applied the tissue of the subject infected with the catalase-positive microbe at a wavelength of 400nm to 460nm, wherein the catalase is inactivated, and wherein the tissue is subsequently to be contacted with a composition comprising a peroxide solution, thereby producing the synergistic antimicrobial effect in the tissue of the subject infected with the catalase-positive microbe. In another example, there is disclosed~~the invention provides~~ a method of producing a synergistic antimicrobial effect in a tissue of a subject infected with a catalase-positive microbe, said method comprising the steps of: applying light to the tissue of the subject infected with the catalase-positive microbe at a wavelength of about 400nm to about 460nm, wherein the catalase is inactivated, and contacting the tissue with a composition comprising a diluted peroxide solution, thereby producing the synergistic antimicrobial effect in the tissue of the subject infected with the catalase-positive microbe.

[0040] In one ~~embodiment~~example, the wavelength is about 410nm.

[0041] In another example~~embodiment~~, the dose of the light is about 5 J/cm^2 to about 200 J/cm^2.

[0042] In yet another example~~embodiment~~, the dose of the light is about 15 J/cm^2.

[0043] In yet another example~~embodiment~~, the catalase-positive microbe is a fungal or bacterial microbe and the light is provided by a pulsed nanosecond laser.

[0044] In yet another example~~embodiment~~, the catalase-positive microbe is a fungal or bacterial microbe and the light is provided by a continuous wave LED.

[0045] In yet another example~~embodiment~~, the diluted peroxide solution is a hydrogen peroxide solution.

[0046] In yet another example~~embodiment~~, the hydrogen peroxide solution is between about 0.03% and about 0.3% hydrogen peroxide.

[0047] In yet another example~~embodiment~~, the method further comprises administering a ROS generating agent to the infected tissue of the subject.

[0048] In yet another example~~embodiment~~, the ROS generating agent is tobramycin, silver cation, iodine tincture, a gold nanoparticle, methylene blue, a β-lactam antibiotic, an aminoglycoside, a fluoroquinolone, an azole, a membrane-targeting polyene antifungal or a cell-wall targeting antifungal.

[0049] In yet another example~~embodiment~~, the tissue is skin, scalp or nails.

[0050] In yet another example~~embodiment~~, the catalase-positive microbe is eradicated.

BRIEF DESCRIPTION OF THE DRAWINGS

[0051] The following Detailed Description, given by way of example, but not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying figures, incorporated herein by reference.

[0052] Figure 1 depicts the effect of ns-410 nm exposure on pure catalase solution. (a). Absorption spectra of pure catalase solution under ns-410 nm exposure. Catalase solution: 3 mg/ml, filtered with a 0.2 μm filter. (b). Percent of remaining active catalase after different treatment schemes (different wavelengths under the same dosage). Quantification of catalase was obtained by an Amplex Red Catalase kit. Data: Mean±standard deviation (N=3).

[0053] Figure 2 depicts the effect of ns-410 nm exposure on active catalase percentages from MRSA USA300 and P. aeruginosa. (a-b). Percent of active catalase remained inside MRSA USA300 (a) and P. aeruginosa (b) after different treatment schemes (different wavelengths under the same dosage). Quantification of catalase was obtained by an Amplex Red Catalase kit. Data: Mean±standard deviation (N=3).

[0054] Figure 3 depicts Resonance Raman spectra of bovine liver catalase powder with and without 410 nm exposure. 410 nm dose: 250 mW/cm2. Raman spectrum acquisition time: 25 s. 532 nm excitation. Data: Mean±SD from five spectra.

[0055] Figure 4 depicts the comparison between ns-410 nm and CW-410 nm exposure on the catalase photoinactivation effect from pure catalase solution (a), catalase from MRSA

CLAIMS

1. Use of a composition comprising a reactive oxygen species (ROS) in the manufacture of a medicament for treating a tissue of a subject infected with a catalase-positive microbe, wherein light is to be applied to the tissue of the subject infected with the catalase-positive microbe at a wavelength of ~~about~~ 400nm to ~~about~~ 430nm, wherein the catalase is inactivated, and the tissue is subsequently to be contacted with a composition comprising a reactive oxygen species (ROS).

2. The use of claim 1, wherein the wavelength is about 410nm.

3. The use of claim 1 or 2, wherein the dose of the light is ~~about~~ 5 J/cm^2 to ~~about~~ 200 J/cm^2, optionally about 15 J/cm^2.

4. The use of any one of claims 1-3, wherein the catalase-positive microbe is a fungal or bacterial microbe and the light is provided by a pulsed nanosecond laser or a continuous wave LED.

5. The use of any one of claims 1-4, wherein the ROS is a hydrogen peroxide solution, optionally wherein the hydrogen peroxide solution is between ~~about~~ 0.03% and ~~about~~ 0.3% hydrogen peroxide, optionally wherein the hydrogen peroxide solution is 0.3% hydrogen peroxide.

6. The use of any one of claims 1-5, wherein the ROS is generated by a ROS generating agent to be administered to the infected tissue of the subject.

7. The use of any one of claims 1-6, wherein the tissue is skin, scalp or nails.

8. A method of disinfecting an inanimate surface contaminated with a catalase-positive microbe, said method comprising the steps of: applying light to the inanimate surface at a wavelength of ~~about~~ 400nm to ~~about~~ 430nm, wherein the catalase is inactivated, and

subsequently contacting the inanimate surface with a composition comprising a reactive oxygen species (ROS), thereby disinfecting the inanimate surface.

9. The method of claim 8, wherein the wavelength is about 410nm.

10. The method of claim 8 or 9, wherein the dose of the light is at least ~~about~~ 5 J/cm^2.

11. The method of any one of claims 8-10, wherein the catalase-positive microbe is a fungus or bacteria and the light is provided by a pulsed nanosecond laser or a continuous wave LED.

12. The method of any one of claims 8-11, wherein the ROS is a hydrogen peroxide solution, optionally wherein the hydrogen peroxide solution is between ~~about~~ 0.03% and ~~about~~ 0.3% hydrogen peroxide, optionally wherein the hydrogen peroxide solution is 0.3% hydrogen peroxide.

13. The method of any one of claims 8-12, wherein the ROS is generated by administering a ROS generating agent to the inanimate surface.

14. The method of any one of claims 8-13, wherein the inanimate surface is a material comprising metal, plastic, fabric, rubber, stone, composite surfaces or wood.

15. Use of a composition comprising a ROS generating agent in the manufacture of a medicament for treating a tissue of a subject infected with a catalase-positive microbe, wherein light from a pulsed nanosecond laser is to be applied to the tissue of the subject infected with the catalase-positive microbe at a wavelength of ~~about~~ 400nm to ~~about~~ 460nm, wherein the catalase is inactivated, and the tissue is subsequently to be contacted with a composition comprising a ROS generating agent.

16. The use of claim 15, wherein the catalase-positive microbe is a fungus or bacteria.

17. The use of claim 15 or 16, wherein the dose of the light is ~~about~~ 5 J/cm^2 – to ~~about~~ 200 J/cm^2, optionally about 15 J/cm^2.

18. The use of any one of claims 15-17, wherein the pulse duration of the light is about 5 nanoseconds.

19. Use of a composition comprising a peroxide solution in the manufacture of a medicament for producing a synergistic antimicrobial effect in a tissue of a subject infected with a catalase-positive microbe, wherein light is to be applied the tissue of the subject infected with the catalase-positive microbe at a wavelength of ~~about~~ 400nm to ~~about~~ 460nm, wherein the catalase is inactivated, and wherein the tissue is subsequently to be contacted with a composition comprising a peroxide solution, thereby producing the synergistic antimicrobial effect in the tissue of the subject infected with the catalase-positive microbe.

20. The use of claim 19, wherein the wavelength is about 410nm.

21. The use of claim 19 or 20, wherein the dose of the light is ~~about~~ 5 J/cm^2 to ~~about~~ 200 J/cm^2, optionally about 15 J/cm^2.

22. The use of any one of claims 19-21, wherein the catalase-positive microbe is a fungal or bacterial microbe and the light is to be provided by a pulsed nanosecond laser or a continuous wave LED.

23. The use of any one of claims 19-22, wherein the peroxide solution is a hydrogen peroxide solution, optionally wherein the hydrogen peroxide solution is between ~~about~~ 0.03% and ~~about~~ 0.3% hydrogen peroxide, optionally wherein the hydrogen peroxide solution is 0.3% hydrogen peroxide.

24. The use of any one of claims 19-23, further comprising ~~administering~~ a ROS generating agent to be administered to the infected tissue of the subject.

25. The use of any one of claims 19-24, wherein the tissue is skin, scalp or nails.

26. The use of any one of claims 1 to 7 or 15 to 25, or the method of any one of claims 8 to 14, wherein the catalase-positive microbe is eradicated.