the use of these dual photosensitizers was found to be ineffective in the absence of dissolved oxygen and light and are ineffective in an aqueous environment.

Well known in the prior art of tumor biology is that malignant cells produce more hydrogen peroxide than normal healthy cells. However, there is a lingering need for improved compositions and therapeutic methods to decompose excess hydrogen peroxide H_2O_2 into hydroxyl radical (ȮH) capable of damaging lipids, proteins, and DNA leading to an ultimate cancer cell death.

Prior art publications indicate a therapeutic method using the Fenton reaction for H_2O_2 decomposition by Fe(II) ions into hydroxyl radical (ȮH). However, prior art synthesis of iron-based therapeutics for use in Fenton reaction is expensive and time-consuming and involves complex purification procedures. In addition, such require special training and expertise in organic and inorganic synthesis. Moreover, in some instances, resulting compositions do not pass toxicity tests due to the inherent toxic nature of the associated ligands. Furthermore, Fenton reactions from prior art iron-based therapeutics form iron-containing sludge ($Fe(OH)_3$) during the course of reaction, which reduce capability for hydroxyl radical production.

Thus, there is continuing need for a non-toxic treatment system, without sludge formation, that is capable of generating reactive oxygen species under various reaction conditions in aqueous solution, which is multifunctional by being active in H_2O_2 rich environments, can be used in aerobic and anaerobic aqueous environments, effective in presence or absence of light, and able to produce non-toxic chemotherapeutic drugs in situ.

Therefore, the long felt need continues for a low cost chemotherapeutic drug solution, available for both developing and developed countries, which can easily be prepared from commercially available chemicals with highest grade of purity, and where little or no special equipment, skills or specialized training are required.

SUMMARY

I have discovered a treatment and diagnostic system by combining features of photodynamic therapy with other anticancer therapeutic methods. This discovery comprises multifunctional treatment and diagnosis systems which comprise at least one reactive oxygen species produced in situ and at least one non-toxic chemotherapeutic agent. Said system slows or stops the progression of bacteria or cancer thus treating various malignancies and bacterial infections. I have found also that variations of said system are fluorophores and function for photodynamic diagnosis.

Variations of these compositions and methods are multifunctional, being capable of producing in situ one or more reactive oxygen species and chemotherapeutic agents under various reactions conditions, such as in aerobic, anaerobic or H_2O_2 rich environments in presence of, or absence of light, being production capable within any of such conditions at the same time or any time or in rapid condition switch sequence from one condition to the other, for illustration, light to dark or aerobic to anaerobic. Variations are further multifunctional in being therapeutic and diagnostic.

In particular variations, I have discovered compositions and methods that produce one or more reactive oxygen species and Juglone based chemotherapeutics in situ. I have found methods for producing hydroxyl radicals in situ in all of (i) aerobic conditions (ii) in anaerobic conditions and (iii) in H_2O_2 rich environments. I also found methods for producing Juglone based chemotherapeutics in situ in all of (i) aerobic conditions (ii) in anaerobic conditions and (iii) in H_2O_2 rich environments. I further found methods for generating singlet oxygen (i) in aerobic conditions and (ii) in anaerobic and aerobic H_2O_2 rich environments.

In one aspect, there is provided a treatment or diagnosis composition comprising

(a) a combination of (1) free base tetrakis *Ar* substituted porphyrine *core* without metal or halide substitution wherein each of the four Ar substituents are at meso positions, are the same, and are selected from an Ar group consisting of any of ortho-, meta-, or para- hydroxyphenyl and alkyl pyridyl, (2) dihydroxynaphthalene or hydroxynaphthalene, (3) hydrated Fe(III) (hMe(III)) and (b) resultant effect of combinations of 1(a)(1),1(a)(2) and 1(a)(3).

In another aspect, there is provided a treatment system comprising 1,5-dihydroxynaphthalene, meso-tetra(N-methyl-4-pyridyl)porphine tetrachloride and Fe(III) chloride in aqueous solution which is multifunctional by producing in situ at least one non-toxic chemotherapeutic agent from a single dose in presence of visible light or in absence of light, effective as treatment composition in both aerobic and anaerobic environments as well as in H_2O_2 rich environment which produces O_2 from excess H_2O_2 when H_2O_2 is present and produces both (a) singlet oxygen and hydroxyl radicals in aerobic conditions and (b) hydroxyl radicals in anaerobic conditions.

In another aspect, there is provided a method to produce one or more reactive oxygen species and Juglone in vitro as resultant effect of combination of free base tetrakis porphyrine core without metal or halide substitution (ArPP), DHN, and hMe(III), wherein optionally mole ratio of hMe(III) is increased or decreased in relation to mole

ratios of ArPP and DHN to selectively produce greater or lesser amount of one reactive oxygen species or Juglone over other resultant effects.

In another aspect, there is provided a method to produce hydroxyl radicals in vitro by combining ArPP, DHN, and hMe(III) in presence of light or absence of light, wherein in absence of light and in anaerobic conditions, hydroxyl radical (ȮH) is produced upon reacting with hydrogen peroxide (H_2O_2).

In another aspect, there is provided a method to produce hydroxyl radicals in vitro in a condition which is aerobic, anaerobic, or H_2O_2 rich environment wherein an H_2O_2 content is at least 200 µM, or any sequence or combination of said conditions, by combining ArPP, DHN, and hMe(III) in presence or absence of light wherein in absence of light and in anaerobic conditions, hydroxyl radical (ȮH) is produced upon reacting combination of ArPP, DHN, and hMe(III) with hydrogen peroxide (H_2O_2).

In another aspect, there is provided a method to produce Juglone chemotherapeutics in vitro in a condition which is aerobic, anaerobic, or H_2O_2 rich environment or any sequence or combination of said conditions, by combining ArPP, DHN, and hMe(III) in presence or absence of light.

In another aspect, there is provided a method to produce singlet oxygen in vitro in a condition which is which is aerobic, anaerobic, or H_2O_2 rich environment wherein an H_2O_2 content is at least 200 µM, or any sequence or combination of said conditions by combining ArPP, DHN, and hMe(III) in presence of light.

In another aspect, there is provided an in vitro method of producing in presence of visible light and in aerobic condition one or more of singlet oxygen ($^1O_2$), hydroxyl radical (ȮH), or Juglone by combining ArPP, DHN, and hMe(III).

In another aspect, there is provided an in vitro method of producing one or more non-toxic chemotherapeutic treatments by combining ArPP, DHN, and hMe(III) by forming at or near mammalian tissue or fluid in one or more regions of treatment (a) in presence of visible light (1) in an aerobic condition singlet oxygen ($^1O_2$), hydroxyl radical (ȮH), and one or more of Juglone, then concurrently or sequentially (2) in an anaerobic condition, hydroxyl radical (ȮH) and Juglone, then forming either concurrently or subsequently forming at or near mammalian tissue or fluid in one or more different regions of treatment (b) in absence of light, (1) hydroxyl radical (ȮH) upon reacting ArPP, DHN, and hMe(III) with hydrogen peroxide (H_2O_2) and one or more of Juglone.

In another aspect, there is provided a tailored composition as defined herein for use in the treatment of tumor hypoxia.

In another aspect, there is provided a portable field treatment kit for preparation and administration of treatment composition mixture for use at remote locations, or for use when rapid preparation and administration are preferred for reasons other than location, comprising (a) visible light resistant durable but flexible package with one or more exterior layers, (b) at least 3 separate sealed, compartments within said exterior layer, with 1 compartment of premeasured quantities of each component ArPP, DHN, and hMe(III), and optionally a compartment for excipient for injection or topical use, all within a single package or assembled separately as a collection of packages, each compartment being breakable upon application of pressure to exterior layers enable combining ArPP, DHN, and hMe(III), with optional excipient in any, to form treatment composition with optional excipient and (c) syringe and needle or other extracting and administering means to extract and inject or topically dispense the treatment composition with optional excipient.

As used in the Specification and Claims,

"DHN" means one or more isomers of dihydroxynaphthalene including the 1,5-form shown in FIG. 1A, 1,5-dihydroxynaphthalene as well as isomers such as 1,x-dihydroxynaphthalene, where x is 2, 3, 4, or 8 or isomers such as 2, x-dihydroxynaphthalene, where x is 3 or 6. Single 'hydroxy' naphthalene reacts similarly to DHN under certain conditions in presence of porphine and metal ions for treatment or diagnostic combinations of this invention, and when so reacting, it is considered a reaction analog within term "DHN";

"Juglone" means 5-hydroxy-1,4-naphthalenedione form as shown in FIG. 1B, but also includes other isomers;

"Derivatives of Juglone" means reaction intermediates or products involving Juglone in reaction path;

"TMPyP" means commercially available free base meso-tetra(N-methyl-4-pyridyl) porphine tetrachloride as shown in FIG. 4A;

"Fe(III)TMPyP" means commercially available Fe(III) bound meso-tetrakis(N-methyl-4-pyridyl) porphyrin as shown in FIG. 4B;

"m-THPP" means commercially available meso-tetra(m-hydroxyphenyl)porphine, also (5,10,15,20-tetrakis(3-hydroxyphenyl)-21H,23H-porphine) in FIG. 4C;

Claims

1. A treatment or diagnosis composition comprising

(a) a combination of

(1) free base tetrakis *Ar* substituted porphyrine *core* without metal or halide substitution wherein each of the four Ar substituents are at meso positions, are the same, and are selected from an Ar group consisting of any of ortho-, meta-, or para-hydroxyphenyl and alkyl pyridyl,

(2) dihydroxynaphthalene or hydroxynaphthalene,

(3) hydrated Fe(III) (hMe(III)) and

(b) resultant effect of combinations of 1(a)(1),1(a)(2) and 1(a)(3).

2. A composition in accordance with claim 1 wherein resultant effect 1(b) is one or more chemotherapeutic therapies selected from group consisting singlet oxygen, hydroxyl radical, and Juglone.

3. A composition in accordance with claim 1 comprising 1(a)(1) selected from one or more of the group consisting of meso-tetrakis(N-methyl-4-pyridyl) porphine tetrachloride, meso-tetrakis(o-hydroxyphenyl)porphine, meso-tetrakis(m-hydroxyphenyl)porphine, and meso-tetrakis(p-hydroxyphenyl)porphine and 1(a)(2) of dihydroxynaphthalene and 1(a)(3) of Fe(III) chloride and 1(b) resultant effect comprising hydroxyl radical.

4. A composition in accordance with claim 1 resulting from the combination of 1(a)(1) of free base meso-tetra(N-methyl-4-pyridyl) porphine tetrachloride (TMPyP) with 1(a)(2) of 1,5-dihydroxynaphthalene (1,5-DHN) and 1(a)(3) of hydrated Fe(III) in initial mole ratios of (i) TMPyP to 1,5-DHN of 1 : 18 to 22, (ii) TMPyP to Fe(III) ions of 1 : 15 to 18.33 and (iii) 1,5-DHN to Fe(III) of 1.1 : 1.3, preferably with initial mole ratios of 1(a)(1) TMPyP to 1(a)(2) 1,5-DHN to 1(a)(3) Fe(III) of 1 : 20 : 16.67, and wherein a final concentration of hydrated Fe(III) is selected to achieve a desired rate of photooxidation of 1,5-DHN by TMPyP.

5. A treatment system comprising 1,5-dihydroxynaphthalene, meso-tetra(N-methyl-4-pyridyl)porphine tetrachloride and Fe(III) chloride in aqueous solution which is

multifunctional by producing in situ at least one non-toxic chemotherapeutic agent from a single dose in presence of visible light or in absence of light, effective as treatment composition in both aerobic and anaerobic environments as well as in H_2O_2 rich environment which produces O_2 from excess H_2O_2 when H_2O_2 is present and produces both (a) singlet oxygen and hydroxyl radicals in aerobic conditions and (b) hydroxyl radicals in anaerobic conditions.

6. A treatment system in accordance with Claim 5 wherein 1(a)(1) is TMPyP, 1(a)(2) is dihydroxynaphthalene (DHN) and 1(a)(3) is Fe(III) ions at mole ratios of 1 TMPyP to 20 DHN to 16.67 for initial Fe(III), and 1(a)(1) is TMPyP as 1.8×10^{-8} moles of TMPyP obtained from (3 mL of 6.0×10^{-6}M), equivalent of 1.47×10^{-5} g (Mwt for TMPyP-818.20), 1(a)(2) is 1,5-DHN present in amount of 3.6×10^{-7} moles obtained from (36 µL of 1.0×10^{-2} M), equivalent to 5.67×10^{-5} g (Mwt for DHN-160.05) and 1(a)(3) is hydrated Fe(III) ions in an initial amount of of 3.0×10^{-7} moles obtained from (30 µL of 1.0×10^{-2} M), equivalent to 4.82×10^{-5} g (Mwt for $FeCl_3$-160.84) but adjustable from the range of 15 µL of 1.0×10^{-2} M to 50 µL of 1.0×10^{-2} M to adjust rate of photooxidation of DHN by TMPyP and 1(b) is one or more chemotherapeutic result effects selected from group consisting singlet oxygen, hydroxyl radical, and Juglone.

7. A method to produce one or more reactive oxygen species and Juglone in vitro as resultant effect of combination of free base tetrakis porphyrine core without metal or halide substitution (ArPP), DHN, and hMe(III), wherein optionally mole ratio of hMe(III) is increased or decreased in relation to mole ratios of ArPP and DHN to selectively produce greater or lesser amount of one reactive oxygen species or Juglone over other resultant effects.

8. A method to produce hydroxyl radicals in vitro by combining ArPP, DHN, and hMe(III) in presence of light or absence of light, wherein in absence of light and in anaerobic conditions, hydroxyl radical (ȮH) is produced upon reacting with hydrogen peroxide (H_2O_2).

9. A method to produce hydroxyl radicals in vitro in a condition which is aerobic, anaerobic, or H_2O_2 rich environment wherein an H_2O_2 content is at least 200 µM, or any sequence or combination of said conditions, by combining ArPP, DHN, and hMe(III) in

presence or absence of light wherein in absence of light and in anaerobic conditions, hydroxyl radical (ȮH) is produced upon reacting combination of ArPP, DHN, and hMe(III) with hydrogen peroxide (H_2O_2).

10. A method to produce Juglone chemotherapeutics in vitro in a condition which is aerobic, anaerobic, or H_2O_2 rich environment or any sequence or combination of said conditions, by combining ArPP, DHN, and hMe(III) in presence or absence of light.

11. A method to produce singlet oxygen in vitro in a condition which is which is aerobic, anaerobic, or H_2O_2 rich environment wherein an H_2O_2 content is at least 200 µM, or any sequence or combination of said conditions by combining ArPP, DHN, and hMe(III) in presence of light.

12. An in vitro method of producing in presence of visible light and in aerobic condition one or more of singlet oxygen ($^1O_2$), hydroxyl radical (ȮH), or Juglone by combining ArPP, DHN, and hMe(III).

13. An in vitro method of producing in absence of light and in anaerobic condition one or more of hydroxyl radical (ȮH) derived from hydrogen peroxide (H_2O_2), or Juglone by combining ArPP, DHN, and hMe(III).

14. An in vitro method of producing one or more non-toxic chemotherapeutic treatments by combining ArPP, DHN, and hMe(III) by forming at or near mammalian tissue or fluid in one or more regions of treatment

(a) in presence of visible light

(1) in an aerobic condition singlet oxygen ($^1O_2$), hydroxyl radical (ȮH), and one or more of Juglone, then concurrently or sequentially

(2) in an anaerobic condition, hydroxyl radical (ȮH) and Juglone,

then forming either concurrently or subsequently forming at or near mammalian tissue or fluid in one or more different regions of treatment

(b) in absence of light,

(1) hydroxyl radical (ȮH) upon reacting ArPP, DHN, and hMe(III) with hydrogen peroxide (H_2O_2) and one or more of Juglone.

15. A tailored composition according to any one of claims 1 to 4 for use in the treatment of tumor hypoxia.

16. A portable field treatment kit for preparation and administration of treatment composition mixture for use at remote locations, or for use when rapid preparation and administration are preferred for reasons other than location, comprising

(a) visible light resistant durable but flexible package with one or more exterior layers,

(b) at least 3 separate sealed, compartments within said exterior layer, with 1 compartment of premeasured quantities of each component ArPP, DHN, and hMe(III), and optionally a compartment for excipient for injection or topical use, all within a single package or assembled separately as a collection of packages, each compartment being breakable upon application of pressure to exterior layers enable combining ArPP, DHN, and hMe(III), with optional excipient in any, to form treatment composition with optional excipient and

(c) syringe and needle or other extracting and administering means to extract and inject or topically dispense the treatment composition with optional excipient.

17. A portable field treatment kit in accordance with Claim 16 wherein preparation of treatment composition mixture and its application can be completed at remote location within one (1) hour or less.