USE OF TARGETED OXIDATIVE THERAPEUTIC FORMULATION IN TREATMENT OF CANCER

BACKGROUND

[0001] This application claims priority to U.S. Provisional Patent Application Serial Number 60/569,695, entitled "Use of Targeted Oxidative Therapeutic Formulation in Treatment of Cancer" filed on May 10, 2004, the entire content of which is hereby incorporated by reference.

[0002] The present invention relates to a composition containing peroxidic species or oxidation products, its method of preparation, and its use. More specifically, the invention relates to a pharmaceutical composition or formulation which contains: peroxidic species or reaction products resulting from oxidation of an olefinic compound, in a liquid form or in a solution, by an oxygen-containing oxidizing agent; a penetrating solvent; a dye containing a chelated metal; and an aromatic redox compound. The invention also relates to the preparation of the pharmaceutical formulation and its use in treating cancer.

[0003] Ozone is a triatomic gas molecule and an allotropic form of oxygen. It may be obtained by means of an electrical discharge or intense ultraviolet light through pure oxygen. The popular misconception that ozone is a serious pollutant, the "free radical" theory of disease, and the antioxidant supplement market have comprehensibly prejudiced medical orthodoxy against its use as a treatment. Ozone therapy, however, is a misnomer. Ozone is an extremely reactive and unstable gas with mechanisms of action directly related to the by-products that it generates through selective interaction with organic compounds present in the plasma and in the cellular membranes. The selective reaction of ozone with unsaturated olefins occurs at the carbon–carbon double bond, generating ozonides. Ozone is toxic by itself, and its reaction products, ozonides, are unstable and are not therapeutic by themselves.

[0004] Hydrogen peroxide (H_2O_2), discovered in 1818, is present in nature in trace amounts. Hydrogen peroxide is unstable and decomposes violently (or foams) when in direct contact with organic membranes and particulate matter. Light, agitation, heating, and iron all accelerate the rate of hydrogen peroxide decomposition in solution. Hydrogen peroxide by direct contact *ex vivo* kills microbes that have low levels of peroxide-destroying enzymes, such as the catalases. However, there is no bactericidal effect when hydrogen peroxide is

infused into the blood of rabbits infected with peroxide-sensitive *E. coli*. Moreover, increasing the concentration of peroxide *ex-vivo* in rabbit or human blood containing *E. coli* produces no evidence of direct bactericidal activity. The lack of effect of high concentrations of hydrogen peroxide is directly related to the presence of the peroxide-destroying enzyme catalase in the host animal's blood. To have any effect, high concentrations of hydrogen peroxide have to be in contact with the bacteria for significant periods of time. Large amounts of hydrogen peroxide-destroying enzymes, such as catalase, normally present in the blood make it impossible for peroxide to exist in blood for more than a few seconds. Thus, hydrogen peroxide introduced into the blood stream by injection or infusion does not directly act as an extracellular germicide in blood or extracellular fluids.

[0005] However, hydrogen peroxide does participate in the bactericidal processes of activated macrophage cells. Activated macrophage cells are drawn to the site of infection or neoplasm, attach to the infectious organism and/or tumor, and ingest them. The killing of the infectious organisms and tumor cells takes place inside the macrophage cell by hydrogen peroxide. Hydrogen peroxide oxidizes cellular chloride to the chlorine dioxide free radical, which destabilizes microbial membranes and, if persistent, induces apoptosis or cellular suicide. The critical therapeutic criteria for intracellular peroxidation are the selective delivery, absorption and activation of peroxidic carrier molecules into only diseased or activated macrophages, which are believed to be incapable of upgraded catalase and glutathione reductase activity. Infused hydrogen peroxide is a generalized poison whereas targeted intracellular peroxidation is a selective therapeutic tool.

[0006] Macrophage cells play critical roles in immunity, bone calcification, vision, neural insulation (myelinization), detoxification, pump strength, and clearance of toxins from the body, depending upon their site of localization. The energy requirements of macrophages are met by intracellular structures called mitochondria. Mitochondria are often structurally associated with the microfilament internal cytoarchitecture. The folded internal layer of the mitochondria creates the high-energy molecule ATP, while the outer layer contains cytochromes and electron recycling molecules that generate peroxides. The outer layers of mitochondria are susceptible to toxic blockade or damage by endotoxins, mycotoxins, virally encoded toxins, drugs, heavy metals, and pesticides. When the peroxidation function of mitochondria is blocked, the filament architecture of the cell tends to cross-link, generating incorrect signals, incompetence, inappropriate replication, or premature cell death.

[0007] The mitochondrial cytochrome oxidase enzyme activity is markedly reduced in many malignant tumors and virus-infected macrophages. (Allen, *et al.*, 1977). In particular, studies of simian viral-transformed and non-transformed cells have shown that the activity of the mitochondrial cytochrome oxidase enzyme in transformed cells was only 50% of the activity in non-transformed cells. (White, *et al.*, 1975). Several studies have also implicated the crosslinking of microfilaments in malignant change, with these tangled microfilaments directly affecting the activity of some oncogenes (Holme, 1990).

[0008] Lymphoma is a broad term encompassing a variety of cancers of the lymphatic system. In lymphoma, cells in the lymphatic system multiply uncontrollably to create a malignant tumor. Lymphoma is differentiated by the cell type and the presentation of the cancerous tumor.

[0009] Lymphomas can develop in virtually any location in the skin. These tumors commonly arise as solitary lesions, but adjacent skin may be at risk for development of the tumor. The gross appearance of lymphoma is quite variable. Early lesions tend to be small superficial nodules that may be covered with normal skin. As the tumor advances, the overlying epidermal layers are destroyed and ulceration, necrosis and a foul odor may be observed as the lesion gradually enlarges.

[0010] Lymphoma is a commonly diagnosed equine tumor, representing up to twenty percent of diagnosed neoplasms. The average age of diagnosis ranges from 8.6 and 14.6 years, but has been reported from as young as 1 year of age to 29 years. UV radiation has been implicated as a contributing factor for development of this tumor and lightly pigmented horses tend to be at an increased risk. Cutaneous carcinomas arise from epidermal cells and are often locally invasive but tend to be slow to metastasize. However, it has been reported that the frequency of metastasis is as high as 18.6%. When metastasis occurs, local lymph nodes are generally the affected sites.

[0011] There are several treatment options, the most appropriate one depending on tumor location. Surgical removal is the most commonly used treatment, wherever possible. Often these tumors manifest in locations where surgical removal is difficult or impossible. Cryosurgery (freezing with liquid nitrogen) and chemotherapy represent alternative treatment options. Depending on the location and size of the tumor, the chemotherapy agent might be administered as a cream or ointment onto the surface of the tumor, or by injection into the

base of the tumor. If diagnosis and treatment are begun early, the prognosis is often positive. However, tumor recurrence is not uncommon within weeks or months later.

[0012] What is needed, therefore, is a method for treating patients affected with cancers such as lymphoma, which is effective and does not produce pronounced side effects.

[0013] U.S. Patent No. 4,451,480 to De Villez teaches a composition and method for treating acne. The method includes topically treating the affected area with an ozonized material derived from ozonizing various fixed oil and unsaturated esters, alcohols, ethers and fatty acids.

[0014] U.S. Patent No. 4,591,602 to De Villez shows an ozonide of Jojoba used to control microbial infections.

[0015] U.S. Patent No. 4,983,637 to Herman discloses a method to parenterally treat local and systemic viral infections by administering ozonides of terpenes in a pharmaceutically acceptable carrier.

[0016] U.S. Patent No. 5,086,076 to Herman shows an antiviral composition containing a carrier and an ozonide of a terpene. The composition is suitable for systemic administration or local application.

[0017] U.S. Patent No. 5,126,376 to Herman describes a method to topically treat a viral infection in a mammal using an ozonide of a terpene in a carrier.

[0018] U.S. Patent No. 5,190,977 to Herman teaches an antiviral composition containing a non-aqueous carrier and an ozonide of a terpene suitable for systemic injection.

[0019] U.S. Patent No. 5,190,979 to Herman describes a method to parenterally treat a medical condition in a mammal using an ozonide of a terpene in a carrier.

[0020] U.S. Patent No. 5,260,342 to Herman teaches a method to parenterally treat viral infections in a mammal using an ozonide of a terpene in a carrier.

[0021] U.S. Patent No. 5,270,344 to Herman shows a method to treat a systemic disorder in a mammal by applying to the intestine of the mammal a trioxolane or a diperoxide

derivative of an unsaturated hydrocarbon which derivative is prepared by ozonizing the unsaturated hydrocarbon dissolved in a non-polar solvent.

[0022] U.S. Patent No. 5,364,879 to Herman describes a composition for the treatment of a medical condition in a mammal, the composition contains a diperoxide or trioxolane derivative of a non-terpene unsaturated hydrocarbon which derivative is prepared by ozonizing below 35° C the unsaturated hydrocarbon in a carrier.

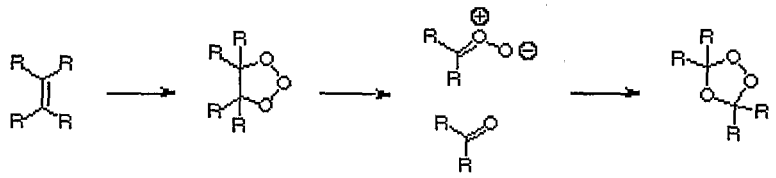
[0023] Despite the reports on the use of terpene ozonides for different medical indications, terpene ozonides display multiple deficiencies. For example, ozonides of monoterpene, such as myrcene and limonene, flamed out in the laboratory. Consequently, they are extremely dangerous to formulate or store.

[0024] Thus, there is a need for a safe and effective pharmaceutical formulation or composition utilizing reaction products from the oxidation of an alkene compound. What is also needed is a method for stimulating mitochondrial defenses against free radical formation and effectively treating individuals affected with cancers such as lymphoma.

SUMMARY

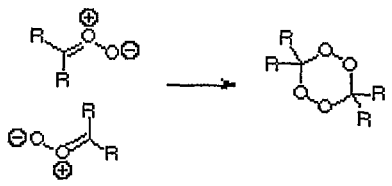
[0025] This invention is directed to pharmaceutical formulations comprising peroxidic species or reaction products resulting from oxidation of an unsaturated organic compound, in a liquid form or in a solution, by an oxygen-containing oxidizing agent; a penetrating solvent; a chelated dye; and an aromatic redox compound. In one embodiment of the pharmaceutical formulation, the essential components include the peroxidic products formed by ozonolysis of an unsaturated alcohol, a stabilizing solvent, metalloporphyrin, and quinone. This invention is also directed to use of the pharmaceutical formulation to treat cancer.

[0026] The peroxidic species or reaction products are preferably formed through the reaction of an alkene and ozone. It is generally accepted that the reaction between an alkene and ozone proceeds by the Criegee mechanism. According to this mechanism, shown in Scheme 1 below, the initial step of the reaction is a 1,3-dipolar cycloaddition of ozone to the alkene to give a primary ozonide (a 1,2,3-trioxalane). The primary ozonide is unstable, and undergoes a 1,3-cycloreversion to a carbonyl compound and a carbonyl oxide. In the absence of other reagents or a nucleophilic solvent, this new 1,3-dipole enters into a second 1,3-dipolar cycloaddition to give the "normal" ozonide, a 1,2,4-trioxalane.



SCHEME 1

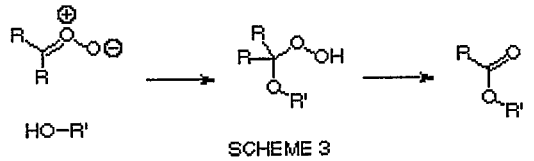
[0027] In a side reaction, the carbonyl oxide can enter into a dimerization to give a peroxidic dimer, the 1,2,4,5-tetraoxane, shown in Scheme 2 below.



SCHEME 2

[0028] The carbonyl oxide is a strongly electrophilic species, and in the presence of nucleophilic species (e.g. alcohols or water), it undergoes facile nucleophilic addition to

give a 1-alkoxyhydroperoxide, shown in Scheme 3 below. Under certain conditions, the 1-alkoxyhydroperoxide can undergo further reaction to give carboxylic acid derivatives.



SCHEME 3

[0029] Again, not wanting to be bound by theory, it is believed that during the ozonolysis of the alcohol-containing alkene in the present invention, it is reasonable to expect that three major types of peroxidic products will be present: the normal ozonide, the carbonyl tetraoxane dimer, and the 1-alkoxyhydroperoxide. In the presence of water, some of these peroxidic products may also lead to the presence of organic peracids in the crude product mixture.

[0030] The present invention also involves the use of a penetrating solvent such as dimethylsulfoxide ("DMSO") to "stabilize" the initial products of the ozonolysis. Similarly, not wanting to be bound by any theory, it is believed that the stabilization is most likely a simple solvation phenomenon. However, DMSO is known to be a nucleophile in its own right. Its participation is also possible as a nucleophilic partner in stabilizing reactive species (for example, as dimethylsulfoxonium salts). The stabilized peroxidic molecule and the penetrating solvent of the current pharmaceutical formulation are made from components generally regarded as safe ("GRAS").

[0031] Another component of the pharmaceutical formulation is a chelated dye, such as a porphyrin. The propensity of metalloporphyrins to sensitize oxygen under photochemical excitation is well documented, as is the propensity of ferroporphyrins and copper porphyrins to bind oxygen-containing systems.

[0032] A further component of the pharmaceutical formulation is an aromatic redox compound, such as a quinone.

[0033] Although not wanting to be bound by any theory, it is postulated that the preferred pharmaceutical formulation is a combination of biochemical agents that induce recycling autocatalytic oxidation in infected, activated, tumorous and dysplastic macrophages.

The pharmaceutical formulation stimulates targeted apoptosis (cell suicide) through unopposed peroxidation. Thus, the pharmaceutical formulation creates therapeutic effects in a number of seemingly disparate mitochondria-based macrophagic diseases. In particular, the pharmaceutical formulation has been shown to selectively kill cancer cells, without collateral damage, in a solid tumor known to have deficient cytochrome oxidase and catalase activity. The pharmaceutical formulation is also effective at reducing tumor cell proliferation and tumor growth. These results indicate its effectiveness at treating cancer.

BRIEF DESCRIPTION OF FIGURES

[0034] Figure 1 shows the nucleotide uptake (^{3}H-TdR) of murine ASL-1 cells treated with different concentrations of one example of the pharmaceutical formulation.

[0035] Figure 2 shows the nucleotide uptake (^{3}H-TdR) of murine ASL-1 cells treated with different concentrations of another example of the pharmaceutical formulation.

[0036] Figure 3 shows the nucleotide uptake (^{3}H-TdR) of murine EL-4 cells treated with different concentrations of one example of the pharmaceutical formulation.

[0037] Figure 4 shows the nucleotide uptake (^{3}H-TdR) of murine EL-4 cells treated with different concentrations of another example of the pharmaceutical formulation.

[0038] Figure 5 shows the number of viable murine ASL-1 cells calculated over time after treatment with different concentrations of one example of the pharmaceutical formulation.

[0039] Figure 6 shows the percentage of dead murine ASL-1 cells calculated at 4 and 20 hours after treatment with either of two examples of the pharmaceutical formulation.

[0040] Figure 7 shows the nucleotide uptake (^{3}H-TdR) of mitogen-stimulated murine lymphocyte cells treated with different concentrations of one example of the pharmaceutical formulation.

[0041] Figure 8 shows the nucleotide uptake (^{3}H-TdR) of mitogen-stimulated murine lymphocyte cells treated with different concentrations of another example of the pharmaceutical formulation.

[0042] Figure 9 shows the nucleotide uptake (^{3}H-TdR) of murine splenocyte tumor cells treated with different concentrations of one example of the pharmaceutical formulation.

[0043] Figure 10 shows the nucleotide uptake (^{3}H-TdR) of murine splenocyte tumor cells treated with different concentrations of another example of the pharmaceutical formulation.

[0044] Figure 11 shows the nucleotide uptake (^{3}H-TdR) of murine thymocyte tumor cells treated with different concentrations of one example of the pharmaceutical formulation.

[0045] Figure 12 shows the nucleotide uptake (^{3}H-TdR) of murine thymocyte tumor cells treated with different concentrations of another example of the pharmaceutical formulation.

[0046] Figure 13 shows the percentage of dead murine thymic lymphoma cells calculated over time after treatment with different concentrations of one example of the pharmaceutical formulation.

[0047] Figure 14 shows the average weights of tumors excised from mice treated with DMSO alone and mice treated with one example of the pharmaceutical formulation.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0048] The current invention pertains to pharmaceutical formulations comprising peroxidic species or reaction products resulting from oxidation of an unsaturated organic compound, in a liquid form or in a solution, by an oxygen-containing oxidizing agent; a penetrating solvent; a chelated dye; and an aromatic redox compound. The pharmaceutical formulations may be used to treat individuals affected with cancers such lymphoma. In one embodiment of the present invention, the essential components of the pharmaceutical formulation include the peroxidic products formed by ozonolysis of an unsaturated alcohol, a stabilizing solvent, metalloporphyrin, and quinone.

[0049] The unsaturated organic compound, which may also be an unsaturated olefinic hydrocarbon, of the pharmaceutical formulation can be an alkene without a hydroxyl group, or a hydroxyl-containing alkene. Preferably, the alkene has less than about 35 carbons. The alkene without a hydroxyl group may be an open-chain unsaturated hydrocarbon, a monocyclic unsaturated hydrocarbon, or a bicyclic unsaturated hydrocarbon. The hydroxyl-containing alkene can be an open-chain unsaturated alcohol, a monocyclic unsaturated alcohol, or a bicyclic unsaturated alcohol. The alkene may also be contained in a fixed oil, an ester, a fatty acid, or an ether.

[0050] Usable unsaturated olefinic hydrocarbons may be unsubstituted, substituted, cyclic or complexed alkenes, hydrazines, isoprenoids, steroids, quinolines, carotenoids, tocopherols, prenylated proteins, or unsaturated fats. The preferred unsaturated hydrocarbons for this invention are alkenes and isoprenoids.

[0051] Isoprenoids are found primarily in plants as constituents of essential oils. While many isoprenoids are hydrocarbons, oxygen-containing isoprenoids also occur such as alcohols, aldehydes, and ketones. In a formal sense, the building block of isoprenoid hydrocarbons may be envisaged as the hydrocarbon isoprene, $CH_2=C(CH_3)-CH=CH_2$, although it is known that isoprene itself is an end-product of isoprenoid biosynthesis and not an intermediate. Isoprenoid hydrocarbons are categorized by the number of isoprene (C_5H_8) units they contain. Thus, monoterpenes have 2, sesquiterpenes have 3, diterpenes have 4, sesterterpenes have 5, triterpenes have 6, and tetraterpenes have 8 isoprene units, respectively. Tetraterpenes are much more commonly regarded as carotenoids.

[0052] Limonene and pinene are examples of a monoterpene. Farnesol and nerolidol are examples of a sesquiterpene alcohol. Vitamin A_1 and phytol are examples of a diterpene alcohol while squalene is an example of a triterpene. Provitamin A_1, known as carotene, is an example of a tetraterpene. Geraniol, a monoterpene alcohol, is liquid in both its oxygen bound and normal states and is safe to living cells.

[0053] Preferred unsaturated hydrocarbons for the pharmaceutical formulation include alkene isoprenoids, such as myricene, citrillene, citral, pinene, or limonene. Preferred unsaturated hydrocarbons also include linear isoprenoid alcohols with two to four repeating isoprene groups in a linear chain, such as terpineol, citronellol, nerol, phytol, menthol, geraniol, geranylgeraniol, linalool, or farnesol.

[0054] The unsaturated organic compound may be linear, branched, cyclic, spiral, or complexed with other molecules in its configuration. The unsaturated organic compound may naturally exist in a gaseous liquid or solid state prior to binding with the oxidizing agent.

[0055] An open-chain unsaturated hydrocarbon can be: C_nH_{2n}, one double bond, n=2-20; C_nH_{2n-2}, two double bonds, n=4-20; C_nH_{2n-4}, three double bonds, n=6-20; C_nH_{2n-6}, four double bonds, n=8-20; $C_{25}H_{40}$, sesterterpene hydrocarbon; or $C_{30}H_{48}$, triterpene hydrocarbon.

[0056] A monocyclic unsaturated hydrocarbon can be: C_nH_{2n-2}, one double bond and one ring, n=3-20; C_nH_{2n-4}, two double bonds and one ring, n=5-20; C_nH_{2n-6}, three double bonds and one ring, n=7-20; $C_{25}H_{40}$, sesterterpene hydrocarbon; or $C_{30}H_{48}$, triterpene hydrocarbon.

[0057] A bicyclic unsaturated hydrocarbon can be: C_nH_{2n-4}, one double bond and two rings, n=4-20; C_nH_{2n-6}, two double bonds and two rings, n=6-20; $C_{25}H_{40}$, sesterterpene hydrocarbon; or $C_{30}H_{48}$, triterpene hydrocarbons.

[0058] An open-chain unsaturated alcohol can be: $C_nH_{2n}O_m$, one double bond, n=3-20, m=1-4; $C_nH_{2n-2}O_m$, two double bonds, n=5-20, m=1-4; $C_nH_{2n-4}O_m$, three double bonds, n=7-20, m=1-4; $C_nH_{2n-6}O_m$, four double bonds, n=9-20, m=1-4; $C_{25}H_{40}O_m$, m=1-4, sesterterpene alcohols; or $C_{30}H_{48}O_m$, m=1-4, triterpene alcohols.

[0059] A monocyclic unsaturated alcohol can be: $C_nH_{2n-2}O_m$, one double bond and one ring, n=3-20, m=1-4; $C_nH_{2n-4}O_m$, two double bonds and one ring, n=5-20, m=1-4; $C_nH_{2n-6}O_m$, three double bonds and one ring, n=7-20, m=1-4; $C_{25}H_{40}O_m$, m=1-4, sesterterpene alcohols; or $C_{30}H_{48}O_m$, m=1-4, triterpene alcohols.

[0060] A bicyclic unsaturate alcohol can be: $C_nH_{2n-4}O_m$, one double bond and two rings, n=5-20, m=1-4; $C_nH_{2n-6}O_m$, two double bonds and two rings, n=7-20, m=1-4; $C_{25}H_{40}O_m$, m=1-4, sesterterpene alcohols; or $C_{30}H_{48}O_m$, m=1-4, triterpene alcohols.

[0061] Based on the total weight of the pharmaceutical formulation, the alkene can vary from about 0.001% to about 30%, preferably from about 0.1% to about 5.0%, and more preferably from about 0.5% to about 3.0%.

[0062] The oxygen-containing oxidizing agent of the pharmaceutical formulation, which oxidizes the unsaturated hydrocarbon, may be singlet oxygen, oxygen in its triplet state, superoxide anion, ozone, periodate, hydroxyl radical, hydrogen peroxide, alkyl peroxide, carbamyl peroxide, benzoyl peroxide, or oxygen bound to a transition element, such as molybdenum (e.g. MoO_5).

[0063] The preferred method to bind "activated oxygen" to intact an isoprenoid alcohol, such as geraniol, is by ozonation at temperatures between 0-20°C in the dark in the absence of water or polar solvent. The geraniol "ozonides" are then dissolved and stabilized in 100% DMSO in the dark to prevent premature breakdown of the products. Although not wanting to be bound by any theory, it is believed that the catalytic breakdown of the tetraoxane peroxidic dimer byproduct of geraniol ozonation, which is not an ozonide, occurs inside of cells in the presence of superoxide anion. The final reactive therapeutic agents released are hydrogen peroxide and acetic acid.

[0064] The pharmaceutical formulation also utilizes a penetrating solvent. The penetrating solvent, which stabilizes the oxygen-bound unsaturated hydrocarbon, may be an emollient, a liquid, a liposome, a micelle membrane, or a vapor. Usable penetrating solvents include aqueous solution, fats, sterols, lecithins, phosphatides, ethanol, propylene glycol, methylsulfonylmethane, polyvinylpyrrolidone, pH-buffered saline, and dimethylsulfoxide ("DMSO"). The preferred penetrating solvents include DMSO, polyvinylpyrrolidone, and pH-buffered saline. The most preferred penetrating solvent is DMSO.

[0065] Based on the total weight of the pharmaceutical formulation, the penetrating solvent can vary from about 50% to about 99%, preferably from about 90% to about 98%, and more preferably from about 95% to about 98%.

[0066] The "stabilized" peroxidic molecule and its penetrating solvent have been made from components currently used in production regulated by the Food and Drug Administration ("FDA"). These ingredients are the subject of Drug Master Files, Drug Monographs, are found in the USP/NF, or are Generally Recognized As Safe ("GRAS").

[0067] Another component of the pharmaceutical formulation is a chelated dye. The dye preferably contains a chelated divalent or trivalent metal, such as iron, copper, manganese tin, magnesium, or strontium. The preferred chelated metal is iron. The propensity of chelated dyes such as metalloporphyrins to sensitize oxygen under photochemical excitation is well documented, as is the propensity of ferroporphyrins and copper porphyrins to bind oxygen-containing systems. Usable dyes include natural or synthetic dyes. Examples of these dyes include porphyrins, rose bengal, chlorophyllins, hemins, porphins, corrins, texaphrins, methylene blue, hematoxylin, eosin, erythrosin, flavinoids, lactoflavin, anthracene dyes, hypericin, methylcholanthrene, neutral red, phthalocyanine, fluorescein, eumelanin, and pheomelanin. Preferred dyes can be any natural or synthetic porphyrin, hematoporphyrin, chlorophyllin, rose bengal, their respective congeners, or a mixture thereof. The most preferred dyes are mixtures of hematoporphyrin and rose bengal and mixtures of hematoporphyrin and chlorophyllin. The dye may be responsive to photon, laser, ionizing radiation, phonon, electrical cardiac impulse, electroporation, magnetic pulse, or continuous flow excitation.

[0068] Based on the total weight of the pharmaceutical formulation or composition, the dye can vary from about 0.1% to about 30%, preferably from about 0.5% to about 5%, and more preferably from about 0.8% to about 1.5%.

[0069] A further component of the pharmaceutical formulation is an aromatic redox compound, such as a quinone. The aromatic redox compound may be any substituted or unsubstituted benzoquinone, naphthoquinone, or anthroquinone. Preferred aromatic redox compounds include benzoquinone, methyl-benzoquinone, naphthoquinone, and methyl-naphthoquinone. The most preferred aromatic redox compound is methyl-naphthoquinone.

[0070] Based on the total weight of the pharmaceutical formulation, the aromatic redox compound can vary from about 0.01% to about 20.0%, preferably from about 0.1% to about 10%, and more preferably from about 0.1% to about 0.5%.

[0071] The pharmaceutical formulation is also preferably activated by an energy source or an electron donor. Useful electron donors include NADH, NADPH, an electrical current, ascorbate or ascorbic acid, and germanium sesquioxide. Preferred electron donors include ascorbate and germanium sesquioxide. The most preferred electron donor is ascorbic acid in any salt form.

[0072] Based on the total weight of the pharmaceutical formulation, the electron donor can vary from about 0.01% to about 20%, preferably from about 1% to about 10%, and more preferably from about 1% to about 5%.

[0073] In order to obtain a biological effect *in vivo,* the pharmaceutical formulation is preferably infused as an ozonolysis-generated peroxidic product of an unsaturated hydrocarbon, rather than an ozonide, in conjunction with a superoxide generating chelated dye and an aromatic quinone. The unsaturated hydrocarbon product, or peroxidic dimer molecule, should be stabilized in a non-aqueous stabilizing solvent and should be capable of penetrating lipid membranes.

[0074] Researchers of energetically activated dye therapy have long known that the superoxide generating dye and the aromatic redox compound preferentially absorb into infected, activated, tumorous and dysplastic cells, which are typically also catalase deficient. Without wanting to be bound by theory, the catalase-induced destruction of peroxide should be overwhelmed in the target cells either naturally or by the pharmaceutical formulation. The peroxidic dimer should also be activated by the superoxide generating dye, initiating electron donation to the dimer and causing the release of hydrogen peroxide and acetic acid intracellularly. The electronic activation of the dye does not always require light, but rather may occur through small electrical pulses provided by, for example, a heart pulse. The peroxidation reaction within the infected macrophage then tends to destroy the prenylated protein linkage of microtubules within the cell, to destroy the infecting toxin, or to induce apoptosis of the macrophage host cell.

[0075] The pharmaceutical formulation is a combination of stable ingredients. These ingredients may preferably be stored as dry solid ingredients and liquid ingredients in separate containers, which are then mixed at the site of use. The dry solid ingredients preferably comprise the chelated dye and the aromatic redox compound. The liquid ingredients preferably comprise the peroxidic species or reaction products resulting from oxidation of the unsaturated hydrocarbon by the oxygen-containing active agent, along with the penetrating solvent. Administration is preferably intravenously. The reconstituted product preferably may be administered intravenously as a concentrate diluted in saline. Rectal, peritoneal and intrathecal deliveries are also possible routes for administration. Intramuscular injection is not preferred, as it has a tendency to produce local irritation.

[0076] Administration of the pharmaceutical formulation *in vivo* is effective in treating neoplasms in affected patients. In particular, the pharmaceutical formulation inhibits both the spontaneous and mitogen-stimulated proliferation of cultured tumor cells, reduces the viable number of cultured tumor cells, and reduces tumor size in vivo. The pharmaceutical formulation has been shown to selectively kill cancer cells, without collateral damage, in a solid tumor known to have deficient cytochrome oxidase and catalase activity.

EXAMPLE 1. OZONOLYSIS OF AN UNSATURATED HYDROCARBON

[0077] Ozonolysis of an alkene may be carried out either in a solvent or neat. In either case, the cooling of the reaction mixture is critical in avoiding explosive decomposition of the peroxidic products of the reaction.

[0078] The following general procedure is typical for the ozonolysis of a liquid alkene.

[0079] A 1-liter flask fitted with a magnetic stirrer is charged with the alkene (2 moles), and the apparatus is weighed. The flask is surrounded by a cooling bath (ice-water or ice-salt). Once the contents are cooled below 5° C, stirring is begun and a stream of ozone in dry oxygen (typically 3% ozone) is passed through the mixture. It is advantageous to disperse the ozonated oxygen through a glass frit, but this is not necessary for a stirred solution. Periodically, the gas stream is stopped, and the reaction flask is weighed or the reaction mixture is sampled. The gas stream is then re-started.

[0080] Once the mass of the reaction flask shows sufficient weight gain, or once the proton magnetic resonance ("H^1 NMR") spectrum of the reaction mixture shows the desired reduction in the intensity of the olefinic proton resonances (usually about 50%), the gas flow is stopped.

[0081] The ozonolysis may be carried out as above, substituting a solution of the alkene in a solvent non-reactive towards ozone such as saturated hydrocarbons or chlorinated hydrocarbons. The ozonolysis may also be carried out as above, with or without solvent, substituting an alkenol for the alkene without affecting the reaction in any substantive manner.

[0082] The reaction mixture is then poured slowly into the cooled penetrating solvent.

EXAMPLE 2. PREPARATION OF THE PHARMACEUTICAL FORMULATION

[0083] A preferred pharmaceutical formulation of the present invention was prepared as follows:

(1) Sparging an ozone/pure oxygen gas mixture of 120 mg/L up through an alkadiene alcohol, 3,7-dimethyl-2,6-octadien-1-ol (geraniol), at 1 Liter of gas per hour;

(2) Maintaining the temperature of the reaction around 5°C;

(3) Removing small aliquots of reaction product hourly and measuring by H^1 NMR the formation of the peroxidic species or reaction products;

(4) Stopping the reaction when more than about 50% of the available unsaturated bonds have been reacted;

(5) Diluting the product mixture with dimethylsulfoxide (1:10) to give a solution or dispersion;

(6) Prior to use in the target biological system, a mixture of hematoporphyrin, rose bengal, and methyl-naphthoquinone dry powders was added to the solution or dispersion in sufficient quantity to create a concentration of 20 micromolar of each component dispersed therein when delivered to the target biological system by saline intravenous infusion. Optionally, ascorbate could be added to the formulation prior to use.

EXAMPLE 3. EXAMPLES OF THE PHARMACEUTICAL FORMULATION

[0084] Two preferred formulations are as follows:

A.

WEIGHT %	INGREDIENT
0.54*	Tetraoxane dimer of acetal peroxide from ozonation of geraniol
98.00	DMSO
0.83	Hematoporphyrin
0.24	Methylnaphthoquinone
0.39	Rose Bengal

*Determined by mass spectroscopy.

B.

WEIGHT %	INGREDIENT
0.54*	Tetraoxane dimer of acetal peroxide from ozonation of geraniol
98.00	DMSO
0.83	Hematoporphyrin
0.24	Methylnaphthoquinone
0.39	Chlorophyllin Sodium-Copper Salt

*Determined by mass spectroscopy.

EXAMPLE 4. QUALITATIVE EVALUATION OF TREATMENT OF EQUINE LYMPHOMA

[0085] The pharmaceutical formulation was injected intravenously into seven subject horses, each affected with a lymphoma tumor cell growth. The dosage and treatment course consisted of 6 treatments of the pharmaceutical formulation, each treatment consisting of 3 cc of Formulation A from Example 3 above in 30 cc normal sterile saline, spaced over a two-week period. Injections were intra-jugular. No other therapeutics or procedures were administered during this treatment course.

[0086] Pre-treatment and follow-up biopsies were performed on all of the subjects, along with a photographic series on one of the subjects. Micrographic and photographic evidence documented resolution of the neoplasia, restoration of normal structures, and eventual healing. Clinical observation noted that over the course of treatments the tumors ashened in color and sloughed off without surgical or other procedural intervention. Post-treatment (30 day) photographic documentation of the subject showed raw granulation and muscle tissue, which was followed by healing with normal epithelial coverage. Final photographic evidence taken two years after initial treatment indicated the continued growth of healthy, asymptomatic tissue. Micrographic documentation also indicated the complete resolution of the tumor and normal tissue re-growth. The remaining subject horses were not photographed but exhibited similar progression of resolution.

EXAMPLE 5. DECREASED PROLIFERATION OF CULTURED TUMOR CELLS

[0087] Two lines of murine tumor cells were cultured separately: ASL-1 (leukemia) and EL-4 (T-cell lymphoma).

[0088] Samples of these cells were treated with Formulation A and Formulation B of Example 3 above, at concentrations of 0%, 0.001%, 0.01%, and 0.1%. To measure the effect the pharmaceutical formulation had on proliferation of the cultured tumor cells, the uptake of tritiated thymidine from the culture medium (^{3}H-TdR) was measured. Uptake of the nucleotide thymidine indicates positive cell proliferation because it demonstrates that the cells are undergoing DNA synthesis.

[0089] The results, shown in Figures 1 – 4, indicate that both Formulation A and Formulation B suppress ASL-1 and EL-4 cell proliferation at concentrations of 0.01% and 0.1%.

[0090] The number of viable ASL-1 cells was also counted after treatment with 0.01% and 0.001% of Formulation A. The results are shown in Figure 5. The number of viable ASL-1 cells was clearly reduced by treatment with Formulation A.

[0091] Figure 6 shows that Formulation A and Formulation B induce apoptosis or cell death in ASL-1 cells. Cell death was not observed at 4 hours after treatment with either

Formulation A or Formulation B. At 20 hours, however, nearly 100% of the ASL-1 cells treated with Formulation A were dead.

[0092] The results indicate that the pharmaceutical formulation is effective at reducing the proliferation of tumor cells and stimulating apoptosis in tumor cells.

EXAMPLE 6. SUPPRESSION OF MITOGEN-STIMULATED LYMPHOCYTE PROLIFERATION

[0093] Murine lympocytes were cultured and treated with a mitogen to stimulate proliferation.

[0094] Samples of these cells were treated with Formulation A and Formulation B of Example 3 above, at concentrations of 0%, 0.001%, 0.01%, and 0.1%. To measure the effect the pharmaceutical formulation had on proliferation of the cultured tumor cells, the uptake of tritiated thymidine from the culture medium (^{3}H-TdR) was measured.

[0095] The results, shown in Figures 7 – 8, indicate that both Formulation A and Formulation B suppress mitogen-stimulated lymphocyte cell proliferation at concentrations of 0.001%, 0.01%, and 0.1%.

EXAMPLE 7. SUPPRESSION OF SPONTANEOUS LYMPHOCYTE PROLIFERATION

[0096] Murine splenocyte and thymocyte tumor cells were cultured.

[0097] Samples of these cells were treated with Formulation A and Formulation B of Example 3 above, at concentrations of 0%, 0.001%, 0.01%, and 0.1%. To measure the effect the pharmaceutical formulation had on proliferation of the cultured tumor cells, the uptake of tritiated thymidine from the culture medium (^{3}H-TdR) was measured.

[0098] The results, shown in Figures 9 – 12, indicate that both Formulation A and Formulation B suppress spontaneous lymphocyte cell proliferation at concentrations of 0.1%, with Formulation A showing suppression at 0.01% as well.

EXAMPLE 8. MORPHOLOGICAL EXAMINATION OF TUMOR CELLS

[0099] Samples of the tumor cells which were killed when treated with the pharmaceutical formulation in Examples 5, 6, and 7 were stained with trypan blue and examined under the microscope. Although the cells appeared dead, they were intact morphologically. This observation indicates that the pharmaceutical formulation induces apoptosis, rather than necrosis, in tumor cells.

EXAMPLE 9. EFFECTS ON CULTURED MURINE THYMIC LYMPHOMAS IN VITRO

[0100] Thymic lymphoma cells were removed from 3-month-old *Atm*-/- mice and cultured.

[0101] Samples of these cells were treated with Formulation A of Example 3 above, at concentrations of 0.01%, and 0.1%, and DMSO. Control samples were treated with DMSO alone.

[0102] Figure 13 shows the percentage of dead tumor cells after treatment with each concentration of Formulation A. The results clearly show that the pharmaceutical formulation reduces the number of viable thymic tumor cells in vitro.

EXAMPLE 10. EFFECTS ON MURINE THYMIC LYMPHOMA CELLS IN VIVO

[0103] Cultured thymic lymphoma cells prepared as in Example 9 (1×10^5) were injected subcutaneously into adult male *Atm*+/+ mice.

[0104] Six days later, the mice were treated with 0.01% concentration of Formulation A of Example 3 through subcutaneous injection at 20 mg/kg body weight. This treatment was given daily for fourteen days. Control mice injected with the cultured lymphoma cells were treated with DMSO. After fourteen days, the tumors growing on the mice were excised and weighed.

[0105] Figure 14 shows the average weight of the tumors in the mice treated with the pharmaceutical formulation and those treated with DMSO. The results indicate that the pharmaceutical formulation suppresses in vivo growth of thymic lymphoma cells, which were cultured and transplanted into immune compromised recipient transgenic mice.

REFERENCES CITED

The following U.S. Patent documents and publications are hereby incorporated by reference.

U.S. Patents

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WHAT IS CLAIMED IS:

1. A method for treating a patient having cancer, comprising:

administering to the patient an effective amount of a pharmaceutical formulation comprising:

peroxidic species or reaction products resulting from oxidation of menthol or an alkene by an oxygen-containing oxidizing agent, wherein the alkene comprises terpineol, citronellol, nerol, linalool, phytol, geraniol, perillyl alcohol, menthol, geranylgeraniol or farnesol, and wherein the peroxidic species or reaction products resulting from oxidation of menthol or the alkene is from about 0.001% to about 30% by weight of the pharmaceutical formulation;

a penetrating solvent, wherein the penetrating solvent comprises dimethylsulfoxide, sterol, lecithin, propylene glycol, or methylsulfonylmethane, and wherein the penetrating solvent is from about 50% to about 99% by weight of the pharmaceutical formulation;

a dye containing a chelated divalent or trivalent metal, wherein the dye comprises porphyrin, rose bengal, chlorophyllin, hemin, corrins, texaphrin, methylene blue, hematoxylin, eosin, erythrosin, lactoflavin, anthracene dye, hypericin, methylcholanthrene, neutral red, phthalocyanine, fluorescein, phthalocyanine, eumelanin, or pheomelanin, and wherein the dye is from about 0.1% to about 30% by weight of the pharmaceutical formulation; and

an aromatic redox compound, wherein the redox compound comprises substituted or unsubstituted benzoquinone, naphthoquinone, or anthroquinone, and wherein the aromatic redox compound is from about 0.01% to about 20% by weight of the pharmaceutical formulation.

2. The method of claim 1, wherein the alkene is in a liquid form, in a solution, or in a dispersion.

3. The method of claim 1, wherein the alkene is contained in a fixed oil, an ester, a fatty acid, or an ether.

4. The method of claim 1, wherein the oxygen-containing oxidizing agent comprises singlet oxygen, oxygen in its triplet state, superoxide anion, periodate, hydroxyl radical, hydrogen peroxide, alkyl peroxide, carbamyl peroxide, benzoyl peroxide, or oxygen bound to a transition element.

5. The method of claim 1, wherein the oxygen-containing oxidizing agent comprises ozone.

6. The method of claim 1, wherein the penetrating solvent is a liquid, micelle membrane, liposome, emollient, or vapor.

7. The method of claim 1, wherein the penetrating solvent is dimethylsulfoxide ("DMSO").

8. The method of claim 1, wherein the dye comprises porphyrin, rose bengal, chlorophyllin, or a mixture thereof.

9. The method of claim 1, wherein the metal comprises iron.

10. The method of claim 1, wherein the metal comprises copper, manganese, tin, magnesium, or strontium.

11. The method of claim 1, further comprising an electron donor.

12. The method of claim 11, wherein the electron donor comprises ascorbic acid or a pharmaceutical salt thereof.

13. The method of claim 1, wherein the cancer is lymphoma.

14. A method for treating a patient having cancer, comprising:

administering to the patient an effective amount of a pharmaceutical formulation comprising:

peroxidic species or reaction products resulting from oxidation of geraniol by a mixture of ozone and oxygen;

dimethylsulfoxide ("DMSO");

a dye containing a chelated divalent or trivalent metal, wherein the dye comprises a mixture of hematoporphyrin and rose bengal or a mixture of hematoporphyrin and chlorophyllin; and

methylnaphthoquinone.

15. The method of claim 14, wherein the cancer is lymphoma.

16. A method for inhibiting the proliferation of tumor cells in a patient, comprising:

administering to the patient an effective amount of a pharmaceutical formulation comprising:

peroxidic species or reaction products resulting from oxidation of menthol or an alkene by an oxygen-containing oxidizing agent, wherein the alkene comprises terpineol, citronellol, nerol, linalool, phytol, geraniol, perillyl alcohol, menthol, geranylgeraniol or farnesol, and wherein the peroxidic species or reaction products resulting from oxidation of menthol or the alkene is from about 0.001% to about 30% by weight of the pharmaceutical formulation;

a penetrating solvent, wherein the penetrating solvent comprises dimethylsulfoxide, sterol, lecithin, propylene glycol, or methylsulfonylmethane, and wherein the penetrating solvent is from about 50% to about 99% by weight of the pharmaceutical formulation;

a dye containing a chelated divalent or trivalent metal, wherein the dye comprises porphyrin, rose bengal, chlorophyllin, hemin, corrins, texaphrin, methylene blue, hematoxylin, eosin, erythrosin, lactoflavin, anthracene dye, hypericin,

methylcholanthrene, neutral red, phthalocyanine, or fluorescein, and wherein the dye is from about 0.1% to about 30% by weight of the pharmaceutical formulation; and

an aromatic redox compound, wherein the redox compound comprises substituted or unsubstituted benzoquinone, naphthoquinone, or anthroquinone, and wherein the aromatic redox compound is from about 0.01% to about 20% by weight of the pharmaceutical formulation.

17. The method of claim 16, wherein the alkene is in a liquid form, in a solution, or in a dispersion.

18. The method of claim 16, wherein the alkene is contained in a fixed oil, an ester, a fatty acid, or an ether.

19. The method of claim 16, wherein the oxygen-containing oxidizing agent comprises singlet oxygen, oxygen in its triplet state, superoxide anion, periodate, hydroxyl radical, hydrogen peroxide, alkyl peroxide, carbamyl peroxide, benzoyl peroxide, or oxygen bound to a transition element.

20. The method of claim 16, wherein the oxygen-containing oxidizing agent comprises ozone.

21. The method of claim 16, wherein the penetrating solvent is a liquid, micelle membrane, liposome, emollient, or vapor.

22. The method of claim 16, wherein the penetrating solvent is dimethylsulfoxide ("DMSO").

23. The method of claim 16, wherein the dye comprises porphyrin, rose bengal, chlorophyllin, or a mixture thereof.

24. The method of claim 16, wherein the metal comprises iron.

25. The method of claim 16, wherein the metal comprises copper, manganese, tin, magnesium, or strontium.

26. The method of claim 16, further comprising an electron donor.

27. The method of claim 26, wherein the electron donor comprises ascorbic acid or a pharmaceutical salt thereof.

28. The method of claim 16, wherein the tumor cells are lymphoma cells.

29. A method for inhibiting the proliferation of tumor cells in a patient, comprising:

administering to the patient an effective amount of a pharmaceutical formulation comprising:

peroxidic species or reaction products resulting from oxidation of geraniol by a mixture of ozone and oxygen;

dimethylsulfoxide ("DMSO");

a dye containing a chelated divalent or trivalent metal, wherein the dye comprises a mixture of hematoporphyrin and rose bengal or a mixture of hematoporphyrin and chlorophyllin; and

methylnaphthoquinone.

30. The method of claim 29, wherein the tumor cells are lymphoma cells.