This invention relates to mediators of apoptosis. Specifically, the invention relates to thiazolium-4-oxo-2(E)-nonenal-glutathione adduct as a novel agent for inducing apoptosis in cells depleted of GSH.

**Intracellular TOG**

**Extracellular TOG**
Figure 1
ENDOGENOUS THIAZADICYCLOGLUTATHIONE-ADDUCT

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority of U.S. Provisional Application Ser. No. 60/853,714 filed Oct. 23, 2006, which is incorporated herein by reference in its entirety.

GOVERNMENT INTEREST

[0002] This invention was supported, in part, by Grant Numbers CA 91016, CA95886, HL70128, and ES013508 from the NIH. The government may have certain rights in the invention.

FIELD OF THE INVENTION

[0003] The invention is directed to mediators of apoptosis. Specifically, the invention relates to thiazadicyclocyclo-4-oxo-2(E)-nonenal-Glutathione adduct as a novel agent for inducing apoptosis in cells depleted of GSH.

BACKGROUND OF THE INVENTION

[0004] Endothelial dysfunction is considered to be the earliest event in atherogenesis. Oxidative stress, inflammation, and apoptosis play critical roles in its progression and onset. Lipid peroxidation, which occurs during oxidative stress, results in the formation of lipid hydroperoxide-derived bifunctional electrophiles such as 4-hydroxy-2(E)-nonenal (HNE) that induce apoptosis.

[0005] Conversely, cancer cells undergo changes which abrogate apoptosis, resulting in immortalization.

[0006] There is a need in the art for finding compounds and markers that will both cause apoptosis and will mark the progression of apoptosis due to pathological processes in the cell.

SUMMARY OF THE INVENTION

[0007] In one embodiment, the invention provides a composition comprising Thiazadicyclo-4-oxo-2(E)-nonenal-Glutathione (TOG).

[0008] In another embodiment, the invention provides a method of inducing apoptosis in a cell comprising the step of contacting said cell with Thiazadicyclo-4-oxo-2(E)-nonenal-Glutathione (TOG), thereby inducing apoptosis in a cell.

[0009] In one embodiment, the invention provides use of Thiazadicyclo-4-oxo-2(E)-nonenal-Glutathione (TOG) in a composition for the treatment of hypoproliferative disease in a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The invention will be better understood from a reading of the following detailed description taken in conjunction with the drawings in which like reference designators are used to designate like elements, and in which:

[0011] FIG. 1 shows intracellular (upper panel) and extracellular (lower panel) TOG in cells treated with dimethyl-TOG (TOG-diester); and

[0012] Dimethyl-TOG causes PARP cleavage in the presence of BSO, acivicin or ONE. The cells were exposed to TOG diester for 8 h. Lysates (50 μg) of the cells were analyzed for PARP cleavage by Western blots. (A) The cells were treated with ONE (positive control) and dimethyl-TOG (dimethyl-TOG) alone, NT (no treatment); (B) The cells were pretreated with BSO for 24 h and then treated with dimethyl-TOG; (C) The cells were pretreated with acivicin for 1 h and then treated with dimethyl-TOG; (D) The cells were treated with dimethyl-TOG together with ONE.

DETAILED DESCRIPTION OF THE INVENTION

[0013] In one embodiment, provided herein are mediators of apoptosis. In another embodiment, provided herein are thiazadicyclocyclo-4-oxo-2(E)-nonenal-Glutathione adducts as novel agents for inducing apoptosis in cells depleted of GSH.

[0014] In one embodiment, the novel thiazadicyclo-4-oxo-2(E)-nonenal-Glutathione (GSH)-adduct, TOG and its analogs described herein are formed in high concentrations in cells treated with ONE and other bifunctional electrophiles while the cells undergo apoptotic cell death. In another embodiment the intracellular to extracellular concentration ratio of TOG and TOG analogs are directly correlated to the rank order of potency for inducing apoptosis by the bifunctional electrophiles. In one embodiment, the GSH-adducts induce biological effects. In another embodiment, the TOG adducts themselves are not detoxification products but mediators of apoptosis. This is supported in another embodiment, by the fact that the toxicity of HNE is increased in cells in which the HNE-GSH-adduct exporter RILP76 is inhibited.

[0015] In one embodiment intracellular TOG, in combination with a GSH depleted environment, causes apoptosis. In another embodiment, formation of TOG and ONO-GSH-adduct causes depletion of GSH; these two factors added together induce apoptosis in another embodiment. In another embodiment, intracellular GSH levels play an important role in apoptosis process. In one embodiment intracellular GSH depletion is not sufficient but necessary to cause apoptosis, especially at the initial stage of apoptosis.

[0016] Accordingly and in one embodiment, provided herein is a composition comprising Thiazadicyclo-4-oxo-2(E)-nonenal-Glutathione (TOG). In another embodiment, the pH of the compositions described herein is equal to 7. In another embodiment, the compositions described herein, which are used in the methods provided, have a pH greater than 7. In one embodiment, the compositions described herein, which are used in the methods provided, further comprise an additional inducer of cell death.

[0017] In one embodiment, the compositions described herein, which are used in the methods provided further comprising at least one of a carrier, excipient, emulsifier, stabilizer, sweetener, flavoring agent, diluent, coloring agent, solubilizing agent or combinations thereof.

[0018] In another embodiment, the compositions described herein, which are used in the methods provided is in the form of a pellet, a tablet, a capsule, a solution, a suspension, a dispersion, an emulsion, an elixir, a gel, an ointment, a cream, or a suppository. In one embodiment, the composition further comprises a carrier, excipient, lubricant, flow aid, processing aid or diluent, wherein said carrier, excipient, lubricant, flow aid, processing aid or diluent is a gum, starch, a sugar, a cellulose material, an acrylate, calcium carbonate, magnesium oxide, talc, lactose monohydrate, magnesium stearate, colloidal silicone dioxide or mixtures thereof.

[0019] In another embodiment, the composition further comprises a binder, a disintegrant, a buffer, a protease inhibitor, a surfactant, a solubilizing agent, a plasticizer, an emul-
sifier, a stabilizing agent, a viscosity increasing agent, a sweetener, a film forming agent, or any combination thereof.

In one embodiment, the compositions described herein, which are used in the methods provided further may be present in the form of suspension or dispersion form in solvents or fats, in the form of a nonionic vesicle dispersion or else in the form of an emulsion, preferably an oil-in-water emulsion, such as a cream or milk, or in the form of an ointment, gel, cream gel, sun oil, solid stick, powder, aerosol, foam or spray.

In one embodiment, the composition is a particulate composition coated with a polymer (e.g., poloxamers or poloxamines). Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral. In one embodiment the pharmaceutical composition is administered parenterally, parentercally, transmucosally, transdermally, intramuscularly, intravenously, intradermally, subcutaneously, intraperitoneally, intravitreally, or intracranially.

In some embodiments, the compositions and methods provided herein permit direct application to the site where it is needed. In the practice of the methods provided herein, it is contemplated that virtually any of the compositions provided herein can be employed.

In one embodiment, the compositions of this invention may be in the form of a pellet, a tablet, a capsule, a solution, a suspension, a dispersion, an emulsion, an elixir, a gel, an ointment, a cream, or a suppository.

In another embodiment, the composition is in a form suitable for oral, intravenous, intraarterial, intramuscular, subcutaneous, parenteral, transmucosal, transdermal, or topical administration. In one embodiment the composition is a controlled release composition. In another embodiment, the composition is an immediate release composition. In one embodiment, the composition is a solid dosage form. In another embodiment, the composition is a liquid dosage form.

In another embodiment, the compositions provided herein are suitable for oral, introral, rectal, parenteral, topical, epicutaneous, transdermal, subcutaneous, intramuscular, intranasal, sublingual, buccal, intradural, intraocular, intrapulmonary, nasal inhalation or a combination thereof. In one embodiment, the step of administering the compositions provided herein, in the methods provided herein is carried out as oral administration, or in another embodiment, the administration of the compositions provided herein is intradural, or in another embodiment, the administration of the compositions provided herein is intrarespiratory, or in another embodiment, the administration of the compositions provided herein is nasal inhalation or in another embodiment, the administration of the compositions provided herein is a combination thereof.

The compounds utilized in the methods and compositions of the present invention may be present in the form of free bases in one embodiment or pharmaceutically acceptable acid addition salts thereof in another embodiment. In one embodiment, the term “pharmaceutically acceptable salts” embraces salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. The nature of the salt is not critical, provided that it is pharmaceutically acceptable. Suitable pharmaceutically acceptable acid addition salts of compounds of Formula I are prepared in another embodiment, from an inorganic acid or from an organic acid. Examples of such inorganic acids are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, alicyclic, heterocyclic, carboxylic and sulfonic classes of organic acids, example of which are formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, 2-hydroxyethanesulfonic, toluenesulfonic, sulfamic, cyclohexylammoniumsulfonic, stearic, algenic, b-hydroxybutyric, salicylic, galactaric and galacturonic acid. Suitable pharmaceutically acceptable base addition salts include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from N,N-dibenzylethylenediamine, chlorpropramine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. All of these salts may be prepared by conventional means from the corresponding compound by reacting, in another embodiment, the appropriate acid or base with the compound.

In one embodiment, the term “pharmaceutically acceptable carriers” includes, but is not limited to, may refer to 0.01-0.1M and preferably 0.05M phosphate buffer, or in another embodiment 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be in another embodiment aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. In one embodiment the level of phosphate buffer used as a pharmaceutically acceptable carrier is between about 0.01 to about 0.1M, or between about 0.01 to about 0.09M in another embodiment, or between about 0.01 to about 0.08M in another embodiment, or between about 0.01 to about 0.07M in another embodiment, or between about 0.01 to about 0.06M in another embodiment, or between about 0.01 to about 0.05M in another embodiment, or between about 0.01 to about 0.04M in another embodiment, or between about 0.01 to about 0.03M in another embodiment, or between about 0.01 to about 0.02M in another embodiment, or between about 0.01 to about 0.015 in another embodiment.
In one embodiment, the compounds of this invention may include compounds modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinylic alcohol, polyvinylpyrrolidone or polyethylene glycol are known to exhibit substantially longer half-lives in blood following intravenous injection than do the corresponding unmodified compounds (Abuschowski et al., 1981; Newmark et al., 1982; and Katre et al., 1987). Such modifications may also increase the compound's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. As a result, the desired in vivo biological activity may be achieved by the administration of such polymer-compound abducts less frequently or in lower doses than with the unmodified compound.

The pharmaceutical preparations comprising the compositions used in one embodiment in the methods provided herein, can be prepared by known dissolving, mixing, granulating, or tablet-forming processes. For oral administration, the active ingredients, or their physiologically tolerated derivatives in another embodiment, such as salts, esters, N-oxides, and the like are mixed with additives customary for this purpose, such as vehicles, stabilizers, or inert diluents, and converted by customary methods into suitable forms for administration, such as tablets, coated tablets, hard or soft gelatin capsules, aqueous, alcoholic or oily solutions. Examples of suitable inert vehicles are conventional tablet bases such as lactose, sucrose, or cornstarch in combination with binders such as acacia, cornstarch, gelatin, with disintegrating agents such as cornstarch, potato starch, algicnic acid, or with a lubricant such as stearic acid or magnesium stearate.

Examples of suitable oily vehicles or solvents are vegetable or animal oils such as sunflower oil or fish-liver oil. Preparations can be effected both as dry and as wet granules. For parenteral administration (subcutaneous, intravenous, intraarterial, or intramuscular injection), the active ingredients or their physiologically tolerated derivatives such as salts, esters, N-oxides, and the like are converted into a solution, suspension, or emulsion, if desired with the substances customary and suitable for this purpose, for example, solubilizers or other auxiliaries. Examples are sterile liquids such as water and oils, with or without the addition of a surfactant and other pharmaceutically acceptable adjuvants. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solutions, and glycols such as propylene glycols or polyethylene glycol are preferred liquid carriers, particularly for injectable solutions.

In addition, the composition described in the embodiments provided herein, can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

An active component can be formulated into the composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide or antibody molecule), which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic; and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, proline, and the like.

In one embodiment, the compositions described herein, which are used in another embodiment, in the methods provided herein, further comprise a carrier, an excipient, a lubricant, a flow aid, a processing aid or a diluent.

The active agent is administered in another embodiment, in a therapeutically effective amount. The actual amount administered, and the rate and time-course of administration, will depend on one embodiment, on the nature and severity of the condition being treated. Prescription of treatment, e.g., decisions on dosage, timing, etc., is within the responsibility of medical practitioners or specialists, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of techniques and protocols can be found in Remington's Pharmaceutical Sciences.

Alternatively, targeting therapies may be used in another embodiment, to deliver the active agent more specifically to certain types of cell, by the use of targeting systems such as antibodies or cell specific ligands. Targeting may be desirable in another embodiment, for a variety of reasons, e.g. if the agent is unacceptably toxic, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

The compositions of the present invention are formulated in one embodiment for oral delivery, wherein the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, algicnic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. Syrup of elixir may contain the active compound sucrose as a sweetening agent methyl and proplyparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. In addition, the active compounds may be incorporated into sustained-release, pulsed release, controlled release or postponed release preparations and formulations.

Controlled or sustained release compositions include formulation in lipophilic depots (e.g. fatty acids, waxes, oils). Also comprehended by the invention are particular compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors.

In one embodiment, the composition can be delivered in a controlled release system. For example, the agent may be administered using intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other
modes of administration. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Rev. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Sauder et al., N. Engl. J. Med. 321:574 (1989). In another embodiment, polymeric materials can be used. In another embodiment, a controlled release system can be placed in proximity to the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)). Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

Such compositions are in one embodiment liquids or lyophilized or otherwise dried formulations and include diluents and various buffer conditions (e.g., Tris-HCl, acetate-phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), solubilizing agents (e.g., glycerol, polyethylene glycol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or toxicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the protein, complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polyactic acid, polyglycolic acid, hydrogels, etc., or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spherosomes. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance. Controlled or sustained release compositions include formulation in lipophilic depots (e.g., fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g., poloxamers or poloxamines). Other embodiments of the compositions of the invention incorporate particulate forms, protective coatings, protease inhibitors, or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal, and oral.

In another embodiment, the compositions of this invention comprise one or more, pharmaceutically acceptable carrier materials. In one embodiment, the carriers for use within such compositions are biocompatible, and in another embodiment, biodegradable. In other embodiments, the formulation may provide a relatively constant level of release of one active component. In other embodiments, however, a more rapid rate of release immediately upon administration may be desired. In other embodiments, release of active compounds may be event-triggered. The events triggering the release of the active compounds may be the same in one embodiment, or different in another embodiment. Events triggering the release of the active components may be exposure to moisture in one embodiment, lower pH in another embodiment, or temperature threshold in another embodiment. The formulation of such compositions is well within the level of ordinary skill in the art using known techniques. Illustrative carriers useful in this regard include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other illustrative post-poned-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (e.g., a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as phospholipids. The amount of active compound contained in

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In one embodiment, within a sustained release formulation depends upon the site of administration, the rate and expected duration of release and the nature of the condition to be treated suppressed or inhibited.

In one embodiment, the compositions described herein, are used in the methods described herein.

In one embodiment, the term “contacting” refers to bringing a subject in contact with the compositions provided herein. For example, in one embodiment, the compositions provided herein are suitable for oral administration, whereby bringing the subject in contact with the composition comprises ingesting the compositions. A person skilled in the art would readily recognize that the methods of bringing the subject in contact with the compositions provided herein, will depend on many variables such as, without any intention to limit the modes of administration; the hemorrhagic event treated, age, pre-existing conditions, other agents administered to the subject, the severity of symptoms, location of the affected area and the like. In one embodiment, provided herein are embodiments of methods for administering the compounds of the present invention to a subject, through any appropriate route, as will be appreciated by one skilled in the art.

In one embodiment, the term “treatment” refers to any process, action, application, therapy, or the like, wherein a subject, including a human being, is subjected to medical aid with the object of improving the subject’s condition, directly or indirectly. In another embodiment, the term “treatment” refers to reducing incidence, or alleviating symptoms, eliminating recurrence, preventing recurrence, preventing incidence, improving symptoms, improving prognosis or combination thereof in other embodiments.

“Treating” embraces another embodiment, the amelioration of an existing condition. The skilled artisan would understand that treatment does not necessarily result in the complete absence or removal of symptoms. Treatment also embraces palliative effects: that is, those that reduce the likelihood of a subsequent medical condition. The alleviation of a condition that results in a more serious condition is encompassed by this term.

In one embodiment, the compositions described herein, are used in the methods provided herein. Accordingly and in one embodiment, provided herein is a method of inducing apoptosis in a cell comprising the step of contacting said cell with Thiazdiaazobicyclo-4-oxo-2(E)-nonenal-Glutathione (TOG), thereby inducing apoptosis in a cell.

In one embodiment, the cell for which the methods and compositions are used to induce apoptosis, is an embryonic cell, or a cancerous cell in another discrete embodiment. In another embodiment, the invention further provides a method of inducing apoptosis in a cell. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising TOG. In another embodiment, the invention provides that TOG induces Poly-ADP-ribose polymerase (PARP) cleavage. In another embodiment, TOG is used as a tumor suppressor. In another embodiment, TOG is used as a cancer suppressor. Each possibility represents a separate embodiment of the present invention.

In one embodiment, “hyperproliferative disease” refers to a disorder characterized by abnormal proliferation of
cells, and generically includes skin disorders such as psoriasis as well as benign and malignant tumors of all organ systems. “Tumor” refers to a neoplasm, and includes both solid and non-solid tumors (such as hematologic malignancies).

In another embodiment, the invention further provides a method of treating a subject affected with a hyper-proliferative disease, comprising the step of administering a composition comprising TOG to a subject, thereby treating a subject affected with a hyper-proliferative disease.

In another embodiment, the invention provides that the hyper-proliferative disease is cancer. In another embodiment, the invention provides that cancer is prostate cancer. In another embodiment, the invention provides that cancer is breast cancer. The compounds are also useful for the prevention or treatment of cancerous and precancerous conditions, including premalignant and malignant hyper-proliferative diseases such as cancers of the breast, skin, prostate, cervix, uterus, colon, bladder, esophagus, stomach, lung, larynx, oral cavity, blood and lymphatic system, metaplasias, dysplasias, neoplasias, leukoplakias and papillomas of the mucous membranes and in the treatment of Kaposi’s sarcoma.

In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising at least 1.2 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 1.2 to 2 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 2 to 10 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 2 to 6 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 4 to 8 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 6 to 10 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 8 to 15 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 10 to 20 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 20 to 40 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 40 to 60 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 60 to 80 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 80 to 100 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 100 to 200 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 150 to 250 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 200 to 300 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 250 to 400 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 400 to 800 μM TOG. In another embodiment, the invention further provides a method of inducing apoptosis in a cell comprising the step of contacting a cell with a composition comprising 600 to 950 μM TOG.

In another embodiment, the invention provides that the cell is comprised within a viable tissue. In another embodiment, the invention provides that the composition comprises TOG attached to a leader compound which directs it to the target cell. In another embodiment, the invention provides that the composition comprises TOG attached to a targeting moiety. In another embodiment, the invention provides that the targeting moiety is tissue specific. In another embodiment, the invention provides that the targeting moiety is cell specific. In another embodiment, the invention provides that the targeting moiety is cell-marker specific. In another embodiment, the invention provides that the targeting moiety is cancerous cell specific. In another embodiment, targeting moieties comprise liposomes, or any other vehicle that can be manipulated to comprise a specific targeting molecule. In another embodiment, liposomes, or any other vehicle that can be manipulated to comprise a specific targeting molecule are known to one of skill in the art. In another embodiment, the specific targeting molecule binds a cancerous antigen. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the invention provides the use of various pharmaceutical nanocarriers. In another embodiment, such carriers provide targeted delivery to the pathological area to provide the maximum therapeutic efficacy. In another embodiment, pharmaceutical nanocarriers of the invention comprise liposomes or polymeric micelles. In another embodiment, the vehicle and/or the targeting moiety minimizes TOG degradation and inactivation upon administration. In another embodiment, the vehicle and/or the targeting moiety prevents undesirable side effects. In another embodiment, the vehicle and/or the targeting moiety increase TOG’s bioavailability and the fraction of drug delivered in the pathological area.

In another embodiment, the vehicle and/or the targeting moiety comprises high-molecular-weight (40 KDa or higher), long-circulating macromolecules that are capable of spontaneous accumulations in various pathological sites, such as solid tumors and infarcted areas, via the so-called enhanced permeability and retention (EPR) effect.

In another embodiment, the vehicle and/or the targeting moiety provides (1) prolonged circulation in the blood and ability to accumulate in various pathological areas (e.g., solid tumors) via the EPR effect (protective polymeric coating with compounds such as polyethylene glycol [PEG] is frequently used for this purpose); and/or (2) the ability to specifically recognize and bind target tissues or cells via the
surface-attached specific ligand (such as but not limited to: monoclonal antibodies as well as their Fab fragments and some other moieties, eg, folate or transferrin, are used for this purpose); and/or (3) the ability to respond to local stimuli characteristic of the pathological site by, for example, releasing an entrapped TOG or specifically acting on cellular membranes under the abnormal pH or temperature in disease sites (this property could be provided by surface-attached pH- or temperature-sensitive components); and/or (4) the ability to penetrate inside cells by bypassing degradation for efficient targeting of intracellular drug targets.

[0055] In another embodiment, the vehicle and/or the targeting moiety comprise an attachment of diagnostic moieties. In another embodiment, the vehicle and/or the targeting moiety are biocompatible. In another embodiment, the vehicle and/or the targeting moiety are non-toxic. In another embodiment, the vehicle and/or the targeting moiety are non-antigenic.

[0056] In another embodiment, to obtain targeted liposomes, many protocols that have been developed to bind corresponding targeting moieties, including antibodies, to the liposome surface without affecting the liposome integrity and antibody properties are known to one of skill in the art.

[0057] In another embodiment, the vehicle and/or the targeting moiety is chemically modified. In another embodiment, the vehicle and/or the targeting moiety is chemically modified with synthetic polymers, such as but not limited to PEG. In another embodiment, the vehicle and/or the targeting moiety provides “steric stabilization” or polymer-mediation protection.

[0058] In another embodiment, TOG is formulated in a stimuli-sensitive nanocarrier. In another embodiment, stimuli-sensitive nanocarriers of the invention can be built releasing the incorporated TOG only when subjected to these “special” conditions of the tumor.

[0059] In another embodiment, TOG is modified with CPPs-proteins and peptides that can facilitate uptake through the cellular membranes thereby enhancing the delivery of TOG inside the cell.

[0060] In another embodiment, the invention provides that apoptosis induced by lipid hydroperoxide-derived bifunctional electrophiles, forms an intracellular glutathione (GSH) adduct derived from ON: with a different mass spectral properties compared to those of the corresponding FREE-GSH adducts.

[0061] In another embodiment, this adduct is TOG. In another embodiment, cells treated with dimethyl-TOG show typical morphological changes of apoptosis, such as detachment from the substrate in one embodiment, or shrinkage, and membrane blebbing in other embodiments, in as early as 1 h after treatment. In one embodiment, the cells treated using the compositions described herein alone, recover and appear normal after 8 h of incubation. In another embodiment, cells treated with 10 μM ON: undergo complete apoptosis. In one embodiment, cells with depleted intracellular GSH, show no recovery and the undergo complete apoptosis under 200 μM TOG diester contact. In another embodiment, pretreatment of cells with acivicin, a gamma-glutamyl transpeptidase (GGT) inhibitor show no recovery and undergo complete apoptosis. In one embodiment, the compositions provided herein further comprise a gamma-glutamyl transpeptidase (GGT) inhibitor. GGT mediates in one embodiment the first step of hydrolysis of extracellular GSH. In another embodiment, the compositions described herein, which are used in the compositions described herein, further comprise an agent capable of depleting extracellular space of Glutathione.

[0062] In one embodiment, provided herein is the use of Thiadiazabicyclo-4-oxo-2(E)-nonenal-Glutathione (TOG) in a composition for the treatment of hyperproliferative disease in a subject in need thereof.

[0063] The term “subject” refers to one embodiment to a mammal including a human in need of therapy for, or susceptible to, a condition or its sequelae. The subject may include dogs, cats, pigs, cows, sheep, goats, horses, rats, and mice and humans. The term “subject” does not exclude an individual that is normal in all respects.

[0064] The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXPERIMENTAL DETAILS SECTION

Materials and Methods

Cell Culture and Treatment

[0065] EA.hy 926 endothelial cells were incubated in DMEM (10 mL) containing 10% FBS, 100 unit/mL of penicillin, and 100 μg/mL of streptomycin at 37°C in an atmosphere of 5% CO2. After the cells reached 70-80% confluence (approximately 2×10⁶ cells), the complete DMEM was removed and replaced with FBS-free DMEM for treatment with dimethyl-TOG.

Preparation of Dimethyl-TOG for Treatment of the Cells

[0066] TOG (10 mg) dissolved in 1 mL of DMF (N,N-Dimethylformamide) was reacted with ethereal diazomethane, which was freshly prepared by reacting N-methyl-N-nitroso-p-toluenesulfonamide with sodium hydride, employing a Diazomethane Generator from Wheaton (Millville, N.J.). The method was used as described by the manufacturer. The reaction was conducted at room temperature for 2 min. The reaction mixture was evaporated to dryness under nitrogen, and dissolved in 1 mL of DMF. The methylation was repeated twice and the reaction mixture was dissolved in acetone/trimethylchlorosilane. TOG could not dissolve in acetone/trimethylchlorosilane and precipitated in the flask. The supernatant was collected and evaporated to dryness to afford the dimethyl ester (5 mg, 50%). High resolution mass spectrometry calculated for C₂₁H₃₃N₃O₈Na (M+Na⁺), 476.1832; found, 476.1848.

Analysis of TOG and Dimethyl-TOG in Dimethyl-TOG Treated Cells

EA.hy 926 Cells

[0067] were incubated until they reached 70-80% confluence (approximately 2×10⁶ cells). The complete DMEM was removed and replaced with FBS-free DMEM for treatment with varying concentrations of ON: or dimethyl TOG dissolved in ethanol. The final concentration of ethanol in the culture medium was <0.1%. FBS was added to the culture medium 1 h after ONE treatment to give a final concentration of 10%. After an additional 30 min of incubation at 37°C, the cell culture medium was collected for LC/MS analysis. The cells were washed with PBS, re-suspended in 300 μL of PBS, lysed by sonication and then filtered through an Amicon Ultra-4 5,000 filter. The cell lysate flow-through and cell media (40 μL of each) were each analyzed in duplicate by LC-MS/MS. The LC System employed a 200×10.0 mm
i.d., 5 µm Phenomenex Jupiter C18 column. Solvent A was 5 mM ammonium acetate in water/0.01% TFA (v/v) and solvent B was 5 mM ammonium acetate in methanol/0.01% TFA (v/v). A linear gradient was run from 3% B at 0 min to 80% B at 33 min, and flow rate was 0.2 mL/min. LC/MS/MS was conducted using a Finnigan TSQ Quantum Ultra AM spectrometer equipped with an ESI source in the positive mode. Operating conditions were as follows: heated capillary temperature 250 °C, spray voltage +5 kV, nitrogen was used as the sheath gas. The auxiliary gas was maintained at 10 (arbitrary units). CID was performed using argon at 1.5 mTorr. Quantification of TOG was performed using peak area ratios for the transition m/z 426 (TOG, M+H)+→m/z 280 (MH+–CONHCH2CO2H–CONH2) compared to the transition m/z 429 (MH+–[H]–TOG internal standard) m/z 283 (MH+–CONHCH2CO2H–CONH2). Intracellular and extracellular TOG concentrations were determined by interpolation from a standard curve prepared by adding a fixed amount of [H]-TOG (10 ng) to increasing amounts of authentic TOG in the blank lysis buffer or incubation medium (range 0.2 ng/mL to 50 ng/mL). A typical regression line for a standard curve of area ratios vs TOG concentrations (ng/mL) in the blank lysis buffer was y=0.065x+0.0045 (r2=0.9999).

Analysis of TOG Methyl Ester-Induced PARP Cleavage

[0068] PARP cleavage was determined by Western blot analysis. Briefly, 50 µg of cellular protein was loaded on pre-cast 7% NuPAGE Novex Tris-Acetate gels and transferred to nitrocellulose membranes. The primary antibody was rabbit anti-human PARP and the secondary antibody was horseradish peroxidase-conjugated goat anti-rabbit antibody.

Example 1
Rate of Reaction Between One and GSH

[0069] To test the hypothesis, that the TOG adds itselfs are not detoxification products but mediators of apoptosis TOG had to be delivered to the cells while monitoring apoptosis through the cleavage poly(ADP-ribose) polymerase (PARP). To increase the membrane permeability of TOG, its dimethyl ester derivative was prepared and was used to treat the EA.hy926 cells. Once the dimethyl-TOG reached the cytosol, the methyl esters were removed by cytosolic esterases to give TOG. A technique based on stable isotope dilution liquid chromatography-multiple reaction monitoring/mass spectrometry (LC-MS/MS) was then employed to monitor the level of intracellular TOG.

[0070] LC-MS analysis of the EA.hy926 cells treated with different concentrations of TOG diester showed no TOG diester in the cells; however, a large amount of TOG diester was found in the extracellular milieu. Quantification of intracellular TOG showed very low levels of TOG in the cells. Indeed, the signal for TOG only appeared when the concentration of TOG diester was higher than 50 µM (Figure A.1). Extracellular TOG amounts were two orders of magnitude higher than those of intracellular TOG. The extracellular signal was detected when the dose of TOG diester was as low as 1 µM. However, the total TOG amount from both the intracellular and extracellular milieu only accounted for approximately 0.02% of the TOG diester added to the cells.

[0071] These data indicate inefficient uptake of the TOG diester into the cells. Meanwhile, intracellular the TOG diester was efficiently hydrolyzed into TOG and exported out of the cells. As the result, there was little accumulation of TOG in the cells. As shown in FIG. 1, with treatment of 200 µM TOG diester, the intracellular TOG level was comparable to those in cells treated with 5 µM ONE. Therefore, high doses of dimethyl-TOG (100 µM and 200 µM) were chosen to detect an apoptosis-inducing effect. When the cells were treated with dimethyl-TOG, typical morphological changes of apoptosis, such as detachment from the substrate, shrinkage, and membrane blebbing were observed as early as 1 h after treatment.

[0072] Surprisingly, the cells would finally recover and appear normal after 8 h of incubation, when most of the cells treated with 100 µM ONE went to complete apoptosis. Western blot analysis showed no sign of PARP cleavage (FIG. 2A). However, if the cells were pretreated with BSO to deplete intracellular GSH, there was no recovery and the cells went to complete apoptosis under 200 µM TOG diester treatment. The same events were observed when cells were treated with actin, a gamma-glutamyltranspeptidase (GGT) inhibitor. GGT mediates the first step of hydrolysis of extracellular GSH. The hydrolyzed amino acids will be transported back to the cells for GSH biosynthesis (4). Without GGT activity, there would be accumulation of GSH in the extracellular medium and depletion of intracellular GSH.

[0073] Interestingly, treatment with low concentrations of ONE had the same effect as BSO and acivicin treatment. ONE at the concentration of 1 µM, showed no apoptosis-inducing effect. However, in combination with 200 µM of TOG diester, apoptosis occurred. It is conceivable that ONE added to the cells extracellularly would first cause damage to proteins on the outside of the cell membrane. GGT, DP and amino acids transporters are all located on the outside of the cell membrane and may serve as excellent targets for ONE-mediated damage.

[0074] It is highly possible that inhibition of GSH-recycling enzymes by ONE has caused intracellular GSH depletion.

[0075] Having described preferred embodiments of the invention with reference to the accompanying drawings, it is to be understood that the invention is not limited to the precise embodiments, and that various changes and modifications may be effected therein by those skilled in the art without departing from the scope or spirit of the invention as defined in the appended claims.

What is claimed:

1. A composition comprising Thiadiazabicyclo-4-oxo-2(E)-monenal-Glutahione (TOG).
2. The composition of claim 1, having pH 7±.7.
3. The composition of claim 1, further comprising an additional inducer of cell death
4. The composition of claim 1, further comprising at least one of a carrier, excipient, emulsifier, stabilizer, sweetener, flavoring agent, diluent, coloring agent, solubilizing agent or combinations thereof.
5. The composition of claim 1, wherein said composition is in the form of a pellet, a tablet, a capsule, a solution, a suspension, a dispersion, an emulsion, an elixir, a gel, an ointment, a cream, or a suppository.
6. The composition of claim 1, wherein said composition is in a form suitable for oral, intravenous, intraarterial, intramuscular, subcutaneous, parenteral, transmuscosal, transdermal, intracranial, or topical administration.
7. The composition of claim 1, wherein said composition is a controlled release composition.
8. The composition of claim 1, wherein said composition is an immediate release composition.

9. The composition of claim 1, wherein said composition is a liquid dosage form.

10. The composition of claim 1, wherein said composition is a solid dosage form.

11. A method of inducing apoptosis in a cell comprising the step of contacting said cell with Thiazabicyclo-4-oxo-2-(E)-nonenal-Glutathione (TOG), thereby inducing apoptosis in a cell.

12. The method of claim 7, wherein said cell is a eukaryotic cell.

13. The method of claim 7, wherein said cell is a cancerous cell.

14. The method of claim 7, wherein said Thiazabicyclo-4-oxo-2-(E)-nonenal-Glutathione (TOG) further induces Poly-ADP-ribose polymerase (PARP) cleavage.

15. A method of treating a hyperproliferative disease in a subject, comprising the step of contacting a cancerous cell of the subject, with the composition of claim 1, thereby inducing apoptosis.

16. The method of claim 11, wherein said hyperproliferative disease is cancer.

17. The method of claim 12, wherein said cancer is a prostate cancer, colon cancer, lung cancer, or pancreatic cancer.

18. The method of claim 11, wherein said cancer is breast cancer.

19. The method of claim 11, whereby contacting is via oral, intravenous, intraarterial, intramuscular, subcutaneous, parenteral, transmucosal, transdermal, intracranial, intratumoral or topical administration.


21. The use of claim 20, whereby the composition further comprises 4-hydroxy-2-(E)-nonenal (HNE), 4-oxo-2-(E)-enal (ONE), or their combination.

22. The use of claim 20, further comprising depleting the subject's hyperproliferating cells of GSH.

23. The use of claim 20, whereby the composition further comprises another agent for the treatment of hyperproliferative disease.

24. The use of claim 20, whereby treating comprises inhibiting, suppressing, reducing incidence, alleviating symptoms, eliminating recurrence, preventing recurrence, delaying incidence, preventing incidence, improving symptoms, improving prognosis, curing or combination thereof.

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