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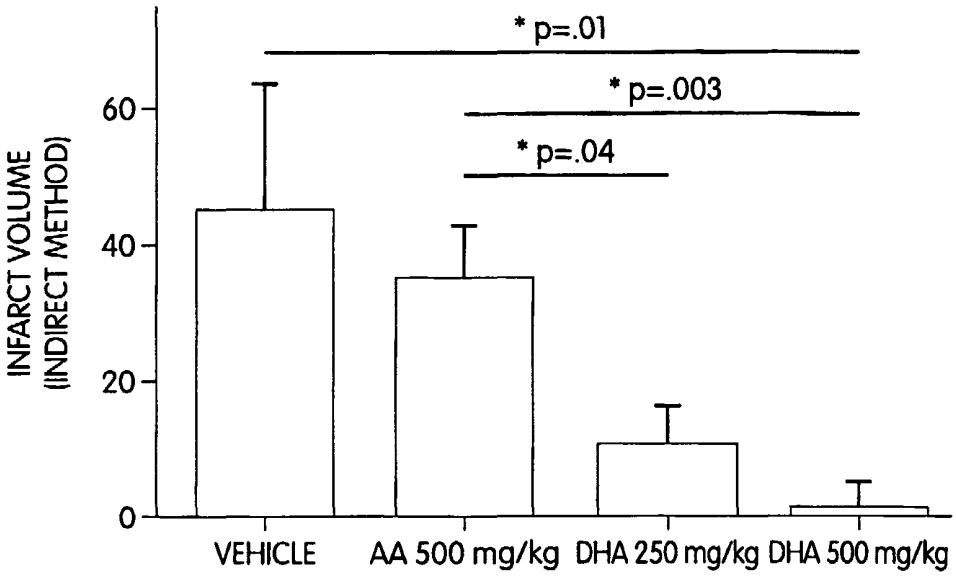
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(54) Title: DEHYDROASCORBIC ACID FORMULATIONS AND USES THEREOF



(57) Abstract: The invention provides improved dehydroascorbic acid compositions and methods for treatment of medical conditions. The dehydroascorbic acid compositions are useful in the treatment of a variety of conditions which benefit from increased dehydroascorbic acid or ascorbic acid concentrations in tissues affected by the conditions.

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DEHYDROASCORBIC ACID FORMULATIONS AND USES THEREOF

Field of the Invention

The invention relates to compositions containing dehydroascorbic acid of reduced toxicity and methods for using such compositions.

Background of the Invention

Ascorbic acid (vitamin C) is an antioxidant which can ameliorate the effects of oxidative free radicals in tissues of the body. Ascorbic acid is poorly transported across cell membrane. Dehydroascorbic acid (DHA), the oxidized form of ascorbic acid, is transported across the cell membrane by glucose transporter (Vera et al., *Nature* 364:79-82, 1993). Once in the cell, dehydroascorbic acid is reduced to ascorbic acid and accumulates in the cell as ascorbic acid (Heath et al., *Exptl. Eye Res.* 5:156-163, 1966; Hammarström, *Acta Physiol. Scand.* 70 Suppl. 289, 1966).

It has been known for some time that ascorbic acid accumulates in the brain, and that dehydroascorbic acid but not ascorbic acid is transported into the brain. Patterson and Mastin described the rapid accumulation of ascorbic acid in the brain following intravenous injection of dehydroascorbic acid but not following injection of ascorbic acid (*Am. J. Physiol.* 167:119-126, 1951). Sjostrand injected ^{14}C-ascorbic acid or ^{14}C-dehydroascorbic acid intravenously in mice and also determined that accumulation of dehydroascorbic acid was considerably greater than ascorbic acid in brain (*Acta Physiol. Scand. Suppl.* 356:1-79, 1970). More recently, Agus et al. have determined that dehydroascorbic acid but not ascorbic acid crosses the blood-brain barrier through glucose transporters (*J. Clin. Invest.* 100:2842-2848, 1997). In PCT application PCT/US98/10608, the same authors have shown that administration of DHA formulated as an unbuffered low pH aqueous solution may be used to increase the levels of ascorbic acid in the brain for the treatment of a variety of neurological disorders. Accumulation of ascorbic acid in heart and liver also were shown to be increased following administration of DHA but not ascorbic acid (Patterson and Mastin, 1951; Sjostrand, 1970), although ascorbic acid levels in the kidney could be increased by administration of either DHA or ascorbic acid (Patterson and Mastin, 1951).

Ascorbic acid typically has a low toxicity regardless of its mode of administration, even at very high doses. Reiser (*Arch. F. Exper. Path. u. Pharmakol.* 190:384-391, 1938) administered ascorbic acid dissolved in 0.9 % unbuffered saline to rabbits (0.2-1.25 g/kg) and

cats (2.5 g/kg). The animals were monitored for 25 hours and no toxicities were observed other than a slight increase in blood pressure in the cats. It also has been determined that the LD50 in rats of intravenous ascorbic acid is >4 g/kg (Hayashi et al., *Pharmacometrics* 12:131, 1976).

In contrast, dehydroascorbic acid is known to have a variety of toxic effects at doses considerably lower than the doses at which ascorbic acid manifests no toxic effects. For example, Patterson (*J. Biol. Chem.* 183:81-88, 1950) showed that rats administered dehydroascorbic acid in unbuffered aqueous solution intravenously at doses of 0.067 - 0.5 g/kg immediately became hyperactive for several minutes and then collapsed. The rats had difficulty breathing which slowly subsided. Not all of the rats recovered from the collapse, however, and this effect depended on the dose administered. One of four rats injected with about 0.25 g/kg died after the collapse. Four of eight rats injected with about 0.33 g/kg died. Four of six rats administered about 0.42 g/kg died after collapsing. The LD50 in rats of unbuffered intravenous dehydroascorbic acid is about 0.32 g/kg (Patterson and Mastin, *Am. J. Physiol.* 167:119-126, 1951). Sjostrand described similar toxic phenomena in rabbits and mice, with hyperactivity, biting, excessive salivation and lacrimation observed following administration of 0.05 - 0.15 mg/kg dehydroascorbic acid but not following administration of ascorbic acid (*Acta Physiol. Scand. Suppl.* 356:1-79, 1970).

Dehydroascorbic acid in aqueous solution is unstable and decomposes at neutral or alkaline pH, but is stable at pH 2-3 (Sjostrand, *Acta Physiol. Scand. Suppl.* 356:1-79, 1970). Sjostrand reported that aqueous dehydroascorbic acid solutions prepared either by oxidation of ascorbic acid as described by Patterson (1950) or from a dehydroascorbic acid-methanol complex had pH values between 2.2 and 2.5.

The proposed uses of dehydroascorbic acid do not take into consideration the well known toxicity of this compound. There is a need, therefore, for compositions and methods useful in the delivery of dehydroascorbic acid to the brain and other structures of the central nervous system, without the toxicity usually associated with dehydroascorbic acid, for the treatment of central nervous system disorders. Other conditions (e.g., those involving oxidative stress) which affect other organs also would benefit from dehydroascorbic acid compositions of reduced toxicity.

Summary of the Invention

It has been discovered that the toxicity of dehydroascorbic acid is unexpectedly and considerably reduced when administered as a buffered formulation. It also has been discovered that buffered formulations of dehydroascorbic acid unexpectedly reduce the effects of ischemia in a well-established mouse model of stroke, even when administered several hours after the ischemic event. Thus the invention includes buffered dehydroascorbic acid compositions and methods of using these compositions in the treatment of various conditions that benefit from increased antioxidant levels, reduced free radicals, or increased ascorbic acid concentrations in cells or tissues.

According to one aspect of the invention, pharmaceutical compositions are provided which include dehydroascorbic acid and a pharmaceutically acceptable buffering system. In preferred embodiments, the compositions have a pH greater than about 3, and a concentration of dehydroascorbic acid of at least about 5 mg/mL. In certain embodiments, the pH is between about 3 and about 7, preferably between about 4 and about 6, more preferably between about 4.5 and about 5.5, or more preferably still between about 4.8 and about 5.2. Most preferably, the pH of the composition is about 5.

In other embodiments the pharmaceutically acceptable buffering system includes an alkaline or buffering agent selected from the group consisting of acetate, phosphate, citrate, glycine, borate, carbonate, bicarbonate, hydroxide and pharmaceutically acceptable salts thereof. Most preferably the alkaline or buffering agent is sodium bicarbonate or sodium acetate.

In still other embodiments, the compositions additionally include a preservative; preferably the preservative is EDTA or sodium benzoate.

In further embodiments, the concentration of dehydroascorbic acid is between about 10 mg/mL and about 1000 mg/mL. Preferably the concentration of dehydroascorbic acid is between about 50 mg/mL and about 750 mg/mL, more preferably between about 100 mg/mL and about 500 mg/mL, and still more preferably between about 200 mg/mL and about 300 mg/mL. Most preferably the concentration of dehydroascorbic acid in the compositions is about 250 mg/mL.

According to another aspect of the invention, methods for treating a subject to increase the concentration of ascorbic acid in a tissue of the subject are provided. The methods include administering to a subject in need of such treatment an amount of a buffered

dehydroascorbic acid composition having a pH greater than about 3 effective to increase the concentration of ascorbic acid in the tissue.

In certain embodiments the buffered dehydroascorbic acid composition is administered by a mode selected from the group consisting of topical, intravenous, oral, intracavity, intrathecal, intrasynovial, buccal, sublingual, intranasal, transdermal, intravitreal, subcutaneous, intramuscular and intrarectal lavage. In certain preferred embodiments the buffered dehydroascorbic acid composition is administered as a bolus intravenous injection or as an intravenous infusion.

In other embodiments the subject has a condition associated with oxidative stress. In certain of these embodiments the oxidative stress results in damage to an epithelial tissue of the subject, wherein the buffered dehydroascorbic acid composition is administered to an apical surface of the epithelial tissue.

In some embodiments the condition associated with oxidative stress is congestive heart failure, atherosclerosis, a neurodegenerative disorder, familial adenomatous polyposis, celiac disease, alcoholic liver disease, inflammatory disease, diabetes, cystic fibrosis, ischemic reperfusion injury, subarachnoid hemorrhage, prion disease, multiple sclerosis, or hyperthyroidism. Preferably the neurodegenerative disorder is Parkinson's disease, Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis, presenile dementia, spongiform encephalopathy, or a behavioral disorders. The inflammatory disease is preferably inflammatory bowel disease , rheumatoid arthritis or pancreatitis. Preferably, the inflammatory bowel disease is Crohn's disease or colitis. In preferred embodiments the prion disease is Creutzfeld-Jakob disease, new variant Creutzfeld-Jakob disease, bovine spongiform encephalopathy or scrapie. The ischemic reperfusion injury preferably is stroke. In other embodiments, the subject has a coronary heart disease.

According to still another aspect of the invention, methods for *ex vivo* preservation of a tissue or organ are provided. The methods include contacting the tissue or organ *ex vivo* with an amount of a buffered dehydroascorbic acid composition having a pH greater than about 3 effective to increase the concentration of ascorbic acid in the tissue or organ. In some embodiments the buffered dehydroascorbic acid composition further includes a standard organ perfusion fluid, such as University of Wisconsin solution, Euro-Collins solution, BT01 solution, Ringer's lactate solution and normal saline solution. In other embodiments, the buffered dehydroascorbic acid composition also includes an organ perfusion agent, such as calcium entry blockers including lidoflazine; cytoprotectors including natriuretic factor, PGI2

and trimetazidine; free radical chelating agents and scavengers including allopurinol, mannitol and glutathione; and substrates for the mitochondrial respiratory chain including aspartate and glutamate.

In another aspect of the invention, methods for treating a condition involving unwanted free radicals are provided. The methods include administering to a cell or tissue of a subject afflicted by such a condition an amount of a buffered dehydroascorbic acid composition having a pH greater than about 3 effective to reduce free radicals in the cell or tissue. Preferred conditions include cancer, cardiovascular disease and cataracts.

Also provided in another aspect of the invention are pharmaceutical compositions which include dehydroascorbic acid and a non-dehydroascorbic acid therapeutic agent, together in an amount effective for treating a condition. In certain embodiments, the pharmaceutical compositions also include a pharmaceutically acceptable buffering system, such that the pharmaceutical compositions has a pH greater than about 3. Preferably the concentration of dehydroascorbic acid is at least about 5 mg/mL.

In certain embodiments of the pharmaceutical compositions, the non-dehydroascorbic agent is one or more antibacterial agents, antifungal agents, antiviral agents, analgesics, non-DHA anti-mucositis agents, antistroke agents, anticancer agents or antineurodegenerative agents. In preferred embodiments, the antistroke agent is an antiplatelet agents, an anticoagulation agents, a thrombolytic agent such as plasminogen activators, an antithrombotic, a neuroprotective agent, a platelet activating factor antagonist, a platelet aggregation inhibitor, a post-stroke and post-head trauma treatment, a cerebral ischemia agent, a basic fibroblast growth factor or a steroid.

According to yet another aspect of the invention, medical products are provided which include an isolated organ in a perfusion fluid containing dehydroascorbic acid. Other medical products provided include an organ perfusion fluid containing dehydroascorbic acid.

In another aspect of the invention, methods are provided for reducing the *in vivo* toxicity of a dehydroascorbic acid pharmaceutical composition. The methods include buffering the dehydroascorbic acid composition to a pH of at least about 3.

According to a further aspect of the invention, methods for reducing the side effects of an anticancer therapeutic treatment are provided. The methods include administering to a subject in need of such treatment an effective amount of a dehydroascorbic acid composition to reduce the side effects, wherein the dehydroascorbic acid composition is administered in combination with the anticancer therapeutic treatment. The dehydroascorbic acid

composition preferably is a buffered dehydroascorbic acid composition having a pH greater than about 3. In certain embodiments the dehydroascorbic acid composition is administered substantially simultaneously with the anticancer therapeutic treatment. In other embodiments the dehydroascorbic acid composition is administered prophylactically prior to the administration of the anticancer therapeutic treatment. The anticancer therapeutic treatment in preferred embodiments is a chemotherapeutic agent.

In still another aspect of the invention, methods for treating tissue injury associated with ulcers of the mouth, pharynx or gastrointestinal tract of a subject are provided. The methods include administering to a subject in need of such treatment an amount of a dehydroascorbic acid composition effective to reduce tissue injury associated with ulcers of the mouth, pharynx or gastrointestinal tract. In preferred embodiments, the dehydroascorbic acid composition is a buffered dehydroascorbic acid composition having a pH greater than about 3 and the concentration of dehydroascorbic acid is at least about 5 mg/mL.

Prior to the present invention, no effective treatment has been available for oral mucositis. The invention involves in another aspect the surprising discovery that the administration of dehydroascorbic acid is efficacious in the treatment of mucositis.

Thus according to another aspect of the invention, methods for treating mucositis in a tissue of a subject are provided. The methods include administering to a subject in need of such treatment an amount of a dehydroascorbic acid composition effective to reduce mucositis in the tissue. In some embodiments the dehydroascorbic acid composition is a buffered dehydroascorbic acid composition having a pH greater than about 3. In other embodiments the mucositis is caused by radiation therapy or chemotherapy. In particular, the methods are useful for the treatment of oral mucositis. Preferably the composition is administered topically.

In other embodiments the methods also include administering to the subject at least one compound selected from the group consisting of antibacterial agents, antifungal agents, antiviral agents, analgesics and non-DHA anti-mucositis agents.

In preferred embodiments of the foregoing methods and compositions, the buffered dehydroascorbic acid composition has a pH between about 3 and about 7, more preferably between about 4 and about 6, still more preferably between about 4.5 and about 5.5, yet more preferably between about 4.8 and about 5.2, and most preferably the pH is about 5.

In preferred embodiments of the foregoing methods and compositions, the concentration of dehydroascorbic acid is between about 5 mg/mL and about 1000 mg/mL,

more preferably between about 50 mg/mL and about 750 mg/mL, still more preferably between about 100 mg/mL and about 500 mg/mL, yet more preferably between about 200 mg/mL and about 300 mg/mL, and most preferably the concentration of dehydroascorbic acid is about 250 mg/mL.

These and other aspects of the invention will be described in further detail in connection with the detailed description of the invention.

Brief Description of the Figures

Fig. 1 depicts reduction of infarct volume as measured by an indirect method.

Fig. 2 shows reduction of infarct volume as determined by a direct (digital) method.

Fig. 3 depicts regional cerebral blood flow following administration of dehydroascorbic acid and ascorbic acid.

Fig. 4 shows that dehydroascorbic acid, but not ascorbic acid, improved neurological scores when administered after ischemia.

Fig. 5 depicts the reduction in mortality of mice having permanent ischemia when administered dehydroascorbic acid compositions.

Detailed Description of the Invention

The invention provides improved dehydroascorbic acid compositions and methods for treatment of medical conditions relating to the administration of dehydroascorbic acid in a buffered composition. The buffered dehydroascorbic acid compositions are useful in the treatment of a variety of conditions which benefit from increased dehydroascorbic acid or ascorbic acid concentrations in tissues affected by the conditions.

The pH of the compositions is controlled by a buffering system. A "buffering system" in a composition, as used herein, is defined as a composition containing buffering agents to regulate the pH of the composition (e.g., solution). Buffering agents suitable for preparation of the compositions of the invention include standard buffer molecules as are known to one of ordinary skill in the art, particularly buffer molecules that are used in pharmaceutical compositions. Preferred buffer molecules include acetate, phosphate, citrate, glycine, borate, carbonate, bicarbonate, hydroxide (and other bases) and pharmaceutically acceptable salts of the foregoing compounds. Particularly preferred buffer molecules include sodium bicarbonate and sodium acetate.

As noted above, buffering agents include molecules having intrinsic buffering capability, as well as bases. In the compositions of the invention, such bases are placed in combination with dehydroascorbic acid to form an acid-base pair. One of ordinary skill in the art can determine by routine experimentation which base molecules and which concentrations will form a buffering system for maintaining dehydroascorbic acid compositions at a desired pH.

The concentration of the buffering agent should be such as to provide adequate buffering of the pH to maintain the pH at a desired target pH, preferably within 0.1 pH unit of the target pH. The target pH of the compositions is greater than about 3 and preferably is between about 3 and about 7. Still more preferably, the pH ranges between about 4 and about 6, between about 4.5 and about 5.5, or between about 4.8 and about 5.2. Most preferably, the pH is about 5.0. The pH values given above are measured at a temperature of about 20°C.

In the ranges given above, it is intended that each pH in the range is embraced by the invention. For example, the range of pH values "between about 3 and about 7" means that pH values of about 3.0, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, . . . , about 6.7, about 6.8, about 6.9 and about 7.0 are included in the range.

When administered to subjects *in vivo*, the compositions of the invention have been found to have much less toxicity than unbuffered solutions of dehydroascorbic acid. Thus, in general, the invention also provides methods for reducing the *in vivo* toxicity of a dehydroascorbic acid pharmaceutical composition by buffering the dehydroascorbic acid composition to a pH of at least about 3. Preferred pH values to which the compositions are buffered are set forth above. Additional components can be added to the compositions as further set forth herein.

The dehydroascorbic acid compositions of the present invention preferably contain at least about 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL, 6 mg/mL, 7 mg/mL, 8 mg/mL, 9 mg/mL or 10 mg/mL of dehydroascorbic acid. In other embodiments, the concentration of dehydroascorbic acid in a composition is at least about 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, or 250 mg/mL. The concentration of dehydroascorbic acid in the present compositions preferably ranges from about 10 mg/mL to about 1000 mg/mL. A more preferred range of dehydroascorbic acid concentration is from about 50 mg/mL to about 750 mg/mL. Still more preferred ranges of concentrations are between about 100 mg/mL and about 500 mg/mL, and between about 200 mg/mL and about 300 mg/mL. Most preferably, the concentration of dehydroascorbic acid is about 250 mg/mL. In the ranges given above, it

is intended that each concentration in the range is embraced by the invention. For example, the range of "between about 200 mg/mL and about 300 mg/mL" means that about 200, about 201, about 202, about 203, about 204, . . . , about 298, about 299 and about 300 mg/mL are included in the range.

The compositions also can include isotonicity agents. An "isotonicity agent" is a compound that is physiologically tolerated and imparts a suitable tonicity to a formulation to prevent the net flow of water across cell membranes that are in contact with the formulation. Compounds, such as glycerin, are commonly used for such purposes at known concentrations. Other possible isotonicity agents include salts (e.g., sodium chloride), dextrose, mannitol, sorbitol, and lactose.

Other additives, such as a pharmaceutically acceptable solubilizers like Tween 20® (polyoxyethylene (20) sorbitan monolaurate), Tween 40® (polyoxyethylene (20) sorbitan monopalmitate), Tween 80® (polyoxyethylene (20) sorbitan monooleate), Pluronic F68® (polyoxyethylene polyoxypropylene block copolymers), and PEG (polyethylene glycol) may optionally be added to the formulation. These additives are not required, but may be useful if the compositions will contact certain materials, e.g. plastics.

The buffered dehydroascorbic acid compositions are useful for treating subjects having conditions which are responsive to increased ascorbic acid or dehydroascorbic acid. In one embodiment, the compositions are used to increase the concentration of ascorbic acid in a cell or tissue, particularly in a cell or tissue of a subject. The compositions may administered systemically or locally in various embodiments, in accordance with the needs of the subjects to which the compositions are given. For example, if the condition being treated is one which is benefited by increasing the concentration of ascorbic acid in the skin, then the compositions are preferably administered topically to the area of the skin which needs treatment. Treatment of specific organs may be carried out by administering the compositions directly (i.e., intraorgan administration). Still other conditions may be affect multiple tissues and therefore administration of the compositions may be advantageously carried out by systemic administration, e.g., intravenously. Thus in general the preferred route of administration can be determined in accordance with standard medical procedures by the practitioner in view of the particular condition being treated.

As described herein, the dehydroascorbic acid compositions are useful in the treatment of various disorders of cells and tissues. As used herein, cells include, but are not limited to, brain cells, neuronal cells, endothelial cells, glial cells, microglial cells, smooth

muscle cells, bone marrow cells, liver cells, intestinal cells, epithelial cells, keratinocytes, fibroblasts, myocytes, mononuclear phagocytes, tumor cells, somatic cells, germ cells and stem cells. Preferred tissues are those which contain the foregoing cells and include brain, gastrointestinal tract, mucosa and skin.

In other embodiments, the compositions are used for treating conditions associated with oxidative stress. Oxidative stress can affect many organ systems, by the induction of protein oxidation or by altering other biochemical mechanisms in cells. It is known that elevated levels of oxidized protein are present in animals and cell cultures following their exposure to various conditions of oxidative stress, including hyperoxia, exercise, ischemia-reperfusion, rapid correction of hyponatremia, paraquat toxicity, magnesium deficiency, ozone, neutrophil activation, cigarette smoking, X-radiation, chronic alcohol treatment, or mixed function oxidation systems (for review, see e.g., Stadtman and Berlett *Chem. Res. Toxicol.* 10, 485-494, 1997). The intracellular level of oxidized protein reflects the balance between the rate of protein oxidation (e.g., generation of reactive oxygen species (ROS)), and the rate of oxidized protein degradation (e.g., by proteases that degrade oxidatively damaged protein). The level of ROS is in part function of the concentration of free radical scavengers (including ascorbic acid).

Oxidative stress also alters the DNA in cells. Experimental and epidemiological evidence suggests that DNA oxidation is mutagenic and contributes to human cancer through several major sources including environmental agents such as tobacco smoke, chronic inflammation, and endogenous oxidants such as leakage from mitochondria. Cigarette smoke contains high levels of NO_x and depletes the body's antioxidants. Elevated DNA oxidation has been observed in association with cancer, for example, during early *Helicobacter pylori* infection, smoking, and exposure to diesel exhaust particles, asbestos, benzene, and aflatoxin.

Oxidative stress, particularly as mediated by ROS such as the superoxide anion (O_2^-), can damage lipids, including those that comprise the cell membrane. Lipid peroxidation has been observed in neurodegenerative diseases, ischemic heart disease, atherosclerosis and alcoholic and nonalcoholic liver disease.

Thus administration of the compositions of the invention is useful in reducing, inhibiting and preventing the effects of oxidative stress by increasing the levels of antioxidants in tissues, cells and/or fluids of a subject. Conditions associated with oxidative stress include congestive heart failure, atherosclerosis, neurodegenerative disorders, familial adenomatous polyposis, celiac disease, alcoholic liver disease, inflammatory diseases

including inflammatory bowel disease (e.g., Crohn's disease; colitis), rheumatoid arthritis, and pancreatitis, diabetes, cystic fibrosis, ischemia-reperfusion injury, prion diseases (Creutzfeldt-Jakob, BSE, scrapie), multiple sclerosis, hyperthyroidism and viral infection associated disorders including oxidative stress in the lung tissue associated with influenza infection and virus-associated hematopoietic disorders, including acquired immune deficiency syndrome from HIV infection.

Further provided in accordance with other embodiments of the invention are methods for treating conditions involving unwanted free radicals. The methods include administering to a cell or tissue of a subject afflicted by such a condition an amount of dehydroascorbic acid effective to reduce free radicals in the cell or tissue. The free radicals can be reduced by increasing the concentration of ascorbic acid or increasing the antioxidant potential in the cell or tissue.

Conditions involving unwanted free radicals include cancer, cardiovascular disease (including atherosclerosis and ischemia/reperfusion conditions) and cataracts (e.g., corneal opacification). Cancers include, but are not limited to, biliary tract cancer, brain cancer (including glioblastomas and medulloblastomas), breast cancer; cervical cancer; choriocarcinoma, colon cancer, endometrial cancer, esophageal cancer, gastric cancer, hematological neoplasms, including acute lymphocytic and myelogenous leukemia, multiple myeloma, AIDS associated leukemias and adult T-cell leukemia lymphoma, intraepithelial neoplasms, including Bowen's disease and Paget's disease, liver cancer, lung cancer, lymphomas, including Hodgkin's disease and lymphocytic lymphomas, neuroblastomas, oral cancer, including squamous cell carcinoma, ovarian cancer, including those arising from epithelial cells, stromal cells, germ cells and mesenchymal cells, pancreatic cancer, prostate cancer, rectal cancer, renal cancer including adenocarcinoma and Wilms tumor, sarcomas, including leiomyosarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma and osteosarcoma, skin cancer, including melanoma, Kaposi's sarcoma, basocellular cancer and squamous cell cancer, testicular cancer, including germinal tumors (seminoma, non-seminoma [teratomas, choriocarcinomas]), stromal tumors and germ cell tumors, and thyroid cancer, including thyroid adenocarcinoma and medullar carcinoma.

In still other embodiments of the invention, the dehydroascorbic acid compositions of the inventions are used in the treatment of subjects with a nervous system disorder. One class of nervous system disorder is the neurodegenerative disorders. "Neurodegenerative disorder" is defined herein as a disorder in which progressive loss of neurons occurs either in the

peripheral nervous system or in the central nervous system. Examples of neurodegenerative disorders include familial and sporadic amyotrophic lateral sclerosis (FALS and ALS, respectively), familial and sporadic Parkinson's disease, Huntington's disease, familial and sporadic Alzheimer's disease, multiple sclerosis, olivopontocerebellar atrophy, multiple system atrophy, progressive supranuclear palsy, diffuse Lewy body disease, corticodentatonigral degeneration, progressive familial myoclonic epilepsy, strionigral degeneration, torsion dystonia, familial tremor, Down's Syndrome, Gilles de la Tourette syndrome, Hallervorden-Spatz disease, diabetic peripheral neuropathy, dementia pugilistica, AIDS dementia, age related dementia, age associated memory impairment, amyloidosis-related neurodegenerative diseases such as those caused by the prion protein (PrP) which is associated with transmissible spongiform encephalopathy (Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker syndrome, scrapie, bovine spongiform encephalopathy and kuru), and those caused by excess cystatin C accumulation (hereditary cystatin C angiopathy), traumatic brain injury (e.g., surgery-related brain injury), cerebral edema, peripheral nerve damage, spinal cord injury, Wernicke-Korsakoff's related dementia (alcohol induced dementia), and presenile dementia. The foregoing examples are not meant to be comprehensive but serve merely as an illustration of the term "neurodegenerative disorder."

Additional nervous system disorders which may be treatable using the dehydroascorbic acid compositions of the invention include neurovascular conditions including stroke, neurological effects of subarachnoid hemorrhage, schizophrenia, behavioral disorders including, but not limited to, dysthymia, involution depression, aggressiveness via dominance, hyperactivity, deprivation syndrome, separation anxiety, intermittent anxiety, instrumental sociopathy, stereotypies, phobia or socialization disorders.

Ischemic conditions are characterized by a stoppage of blood flow to a tissue or organ. The stoppage may result from a blockage in the blood vessel supplying the tissue or organ (e.g. a stroke), or may result when the heart stops beating (e.g. a heart attack). Reperfusion is the term which describes the restarting of the supply of blood to the organ or tissue following ischemia.

The present invention in another aspect utilizes the unexpected finding that buffered dehydroascorbic acid compositions can be used to reduce or prevent damage due to ischemia and/or reperfusion. The buffered DHA compositions can be administered prior to an ischemic event (i.e., prophylactically), or after an ischemic event has occurred (i.e., acutely).

When used in the treatment of stroke, for example, the buffered DHA compositions of the invention unexpectedly reduced the infarct volume, lowered mortality, and increased cerebral blood flow, even if administered as long as 3 hours after a stroke.

Accordingly the present invention is useful whenever it is desirable to prevent, inhibit altogether or reduce damage due to ischemia and/or reperfusion of tissue. The invention thus is useful in the treatment of ischemia, particularly in the prophylactic treatment of ischemia. In particular, the methods of treatment disclosed herein can be used to reduce brain injury resulting from strokes and/or perioperative ischemia during neural surgery. The methods of treatment disclosed herein also can be used to reduce tissue injury resulting from ischemia in other organs including heart, kidney, pancreas, lung, intestine and the like.

In further embodiments of the invention, the dehydroascorbic compositions can be used in the treatment of cardiovascular disease, including ischemic heart disease, congestive heart failure and atherosclerosis.

In additional embodiments of the invention, the dehydroascorbic acid compositions described herein are useful for treatment of disorders of the mucosa and epithelial linings of body cavities. In particular, such disorders include those affecting the gastrointestinal tract, such as mucositis, stomatitis, xerostomia, esophagitis, enteritis, gastritis, bowel inflammation (e.g., colitis), and ulcer formation in various organs of the mouth, pharynx and gastrointestinal tract.

For example, oral ulcerative mucositis is a common, painful, dose-limiting toxicity of drug (chemotherapy) and radiation therapy for cancer. The disorder is characterized by breakdown of the oral mucosa that results in the formation of ulcerative lesions. In granulocytopenic patients, the ulcerations that accompany mucositis are frequent portals of entry for indigenous oral bacteria often leading to sepsis or bacteremia. Mucositis occurs to some degree in more than one third of patients receiving anti-neoplastic drug therapy. The frequency and severity are significantly greater among patients who are treated with induction therapy for leukemia or with many of the conditioning regimens for bone marrow transplant. Among these individuals, moderate to severe mucositis is not unusual in more than three-quarters of patients. Moderate to severe mucositis occurs in virtually all patients who receive radiation therapy for tumors of the head and neck and typically begins with cumulative exposures of 15 Gy and then worsens as total doses of 60 Gy or more are reached.

The disorders of the mucosa and epithelial linings may be quite complex and many tissues and cell types may be affected. For example, oral mucositis appears to represent a

sequential interaction of oral mucosal cells and tissues including connective tissue, endothelium, epithelium, inflammatory cells, pro-inflammatory cytokines and local environmental factors such as bacteria and saliva. Damage to epithelial and connective tissue induces free radical formation, the release of pro-inflammatory cytokines (TNF-α, IL-1) and local tissue injury. Additionally, both direct and indirect effects of therapy on epithelial cells results in either apoptotic or necrotic changes of basal epithelial cells; differentiation into renewing epithelium ceases and is followed by atrophy and ulceration.

Clinically mucositis progresses through three stages. In the first stage, inflammation is accompanied by painful mucosal erythema, which can respond to local anesthetics. The second stage is characterized by painful ulceration with pseudomembrane formation and, in the case of myelosuppressive treatment, potentially life-threatening sepsis, requiring antimicrobial therapy. Pain is often of such intensity as to require parenteral narcotic analgesia. The third stage includes spontaneous healing, occurring about 2 - 3 weeks after cessation of anti-neoplastic therapy.

Standard therapy for mucositis is predominantly palliative, including application of topical analgesics such as lidocaine and/or systemic administration of narcotics and antibiotics. Currently, there is no approved treatment for mucositis and accordingly there is a need for efficacious methods and compositions for the treatment of mucositis.

The invention includes methods for treatment of mucositis and other disorders of mucous membranes which include administration of dehydroascorbic acid compositions. In contrast to the well-known toxicities of intravenously administered dehydroascorbic acid, topical and oral administration of DHA is not associated with severe toxicity. Accordingly, treatment of mucositis can use buffered or unbuffered dehydroascorbic acid compositions. Preferably, the dehydroascorbic acid compositions used are buffered and formulated as described elsewhere herein. Compositions useful for the treatment of mucositis also preferably are buffered as described herein, although unbuffered compositions can be used if desired.

Additional embodiments of the invention provide methods for the treatment of dental indications, including gingivitis, calculus formation and caries and skin disorders, including dermatitis, age-associated skin changes and UV photodamage. The method include the administration of dehydroascorbic acid compositions to the affected tissue.

Prophylactic or acute administration of the dehydroascorbic acid compositions of the invention is contemplated for treatment of the disorders described herein. Thus the DHA

compositions can be administered as part of a preventive regimen and/or in response to onset of a particular condition.

The invention also provides combination therapies wherein an effective amount of dehydroascorbic acid is administered in conjunction with other therapeutic agents for the particular condition being treated. The administration of dehydroascorbic acid and the other therapeutics may be performed concurrently (with the therapeutic agents mixed together or as separate compositions), sequentially or at different time points.

For example, when treating Alzheimer's Disease, the therapeutic agents which can be combined with dehydroascorbic acid include, but are not limited to, estrogen, vitamin E (alpha-tocopherol), Tacrine (tetrahydroacridinamine), selegiline (deprenyl), and Aracept (donepezil).

As another example, when treating Parkinson's disease, the therapeutic agents which can be combined with dehydroascorbic acid include, but are not limited to, the anticholinergic class of drugs, clozapine, levodopa with carbidopa or benserazide, pergolide mesylate, selegiline (deprenyl), pramipexole, and dopamine agonist class of drugs.

One of ordinary skill in the art will be familiar with additional therapeutic agents useful in combination with dehydroascorbic acid for the treatment of various conditions as described herein. See, for example, U.S. Patents 5,670,477 and 5,735,814 for therapeutics useful for the treatment of neurodegenerative disorders (i.e., antineurodegenerative agents).

In other embodiments, combinations of dehydroascorbic acid and other therapeutic agents can be used in the treatment of ischemia-reperfusion injuries. For example, compositions having dehydroascorbic acid and one or more antistroke agents can be prepared for administration to subjects having a need for such treatment. One of ordinary skill in the art is familiar with a variety of antistroke agents which are used in the medical arts to treat stroke (thrombotic, embolic and/or hemorrhagic stroke). Such agents include antiplatelet agents, anticoagulation agents, thrombolytic agents including plasminogen activators, antithrombotics, neuroprotective agents, platelet activating factor antagonists, platelet aggregation inhibitors, post-stroke and post-head trauma treatments, cerebral ischemia agents, basic fibroblast growth factors and steroids.

Antiplatelet agents, which inhibit platelet aggregation, include aspirin, ticlopidine and dipyridamole.

Anticoagulation agents reduce or prevent the coagulation of blood components and thus reduce or prevent clot formation; common anticoagulation agents include coumarin and heparin.

Thrombolytic agents function by lysing the clot which causes the thromboembolic stroke. Commonly used thrombolytic agents include urokinase, streptokinase and tissue plasminogen activator (alteplase, tPA). Various modified forms of tPA ("modified tPA") have been characterized and are known to those skilled in the art. Modified tPA includes, but is not limited to, variants having deleted or substituted amino acids or domains, variants conjugated to other molecules, and variants having modified glycosylation.

Antithrombotics include anagrelide hydrochloride; bivalirudin; dalteparin sodium; danaparoid sodium; dazoxiben hydrochloride; efegatran sulfate; enoxaparin sodium; ifetroban; ifetroban sodium; tinzaparin sodium; and trifenagrel.

Neuroprotective agents include dizocilpine maleate.

Platelet activating factor antagonists include lexipafant.

Platelet aggregation inhibitors include acadesine; beraprost; beraprost sodium; ciprostene calcium; itazigrel; lifarizine; oxagrelate.

Post-stroke and post-head trauma agents include citicoline sodium and nimodipine.

Cerebral ischemia agents include dextrorphan hydrochloride.

Treatment of cancer in a patient with radiation therapy and/or chemotherapy can induce multiple side effects in the patient. For example, a well known set of side effects of radiation therapy is the induction of oral mucositis and oral candidiasis in the patient. It is known in the art that prophylactic use of antifungal agents can reduce the clinical signs of oral candidiasis. Accordingly, combinations of dehydroascorbic acid and other therapeutic agents also can be used to reduce multiple side effects radiation therapy and/or chemotherapy. As shown herein, dehydroascorbic acid is therapeutically effective in lessening the severity and reducing the duration of mucositis. Other therapeutic agents that can be combined advantageously with dehydroascorbic acid include anti-fungal agents, analgesics, antimicrobials, anticancer agents and non-DHA anti-mucositis agents. The therapeutics for mucositis treatment preferably are administered orally, in liquid form or as a mucosa-adhesive water-soluble polymer film (e.g., Oguchi et al., *Int. J. Radiat. Oncol. Biol. Phys.* 40(5):1033-1037, 1998).

Antimucositis agents (other than DHA as disclosed herein) include clarithromycin (Woo et al., *Pharmacol. Res.* 41(5):527-532, 2000), glutamine (Huang et al., *Int. J. Radiat.*

Oncol. Biol. Phys. 46(3):535-539, 2000), GM-CSF (Ibrahim et al., *Med. Oncol.* 14(1):47-51, 1997), sucralfate (Cengiz et al., *J. Clin. Gastroenterol.* 28(1):40-43, 1999), pentoxifylline (Bianco et al., *Blood* 78(5):1205-1211, 1991), G-CSF (filgrastim; Karthaus et al., *Bone Marrow Transplant.* 22(8):781-785, 1998), tretinoin (Cohen et al., *Oral Dis.* 3(4):243-246, 1997), chlorhexidine digluconate (Ferretti et al., *Oral Surg. Oral Med. Oral Pathol.* 69(3):331-338, 1990), Azelastine (Osaki et al., *Head Neck* 16(4):331-339, 1994), benzydamine, dinoprostone (prostaglandin E2), silver nitrate, and beta-carotene (Verdi, *Drug Saf.* 9(3):185-195, 1993), interleukin-11 (IL-11; Sonis et al., *Eur. J. Cancer B Oral Oncol.* 31B(4):261-266, 1995), povidone-iodine (Adamietz et al., *Support Care Cancer.* 6(4):373-377, 1998)

Antifungal agents include acrisorcin; ambruticin; amphotericin B; azaconazole; azaserine; basifungin; bifonazole; biphenamine hydrochloride; bispyrithione magsulfex; butoconazole nitrate; calcium undecylenate; candicidin; carbol-fuchsin; chlordantoin; ciclopirox; ciclopirox olamine; cilofungin; cisconazole; clotrimazole; cuprimyxin; denofungin; dipyrithione; doconazole; econazole; econazole nitrate; enilconazole; ethonam nitrate; fenticonazole nitrate; filipin; fluconazole; flucytosine; fungimycin; griseofulvin; hamycin; isoconazole ; itraconazole; kalafungin; ketoconazole; lomofungin; lydimycin; mepartricin; miconazole; miconazole nitrate; monensin ; monensin sodium ; naftifine hydrochloride; neomycin undecylenate; nifuratel; nifurmerone; nitralamine hydrochloride; nystatin; octanoic acid; orconazole nitrate; oxiconazole nitrate; oxifungin hydrochloride; parconazole hydrochloride; partricin; potassium iodide ; proclono ; pyrithione zinc ; pyrrolnitrin; rutamycin; sanguinarium chloride; saperconazole; scopafungin; selenium sulfide; sinefungin; sulconazole nitrate; terbinafine; terconazole; thiram; ticlatone ; tioconazole; tolciclate; tolindate; tolnaftate; triacetin; triafungin; undecylenic acid; viridofulvin; zinc undecylenate; zinoconazole hydrochloride.

Other antimicrobials include antibiotic agents such as acedapsone; acetosulfone sodium; alamecin; alexidine; amdinocillin; amdinocillin pivoxil; amicycline; amifloxacin; amifloxacin mesylate; amikacin; amikacin sulfate; aminosalicylic acid; aminosalicylate sodium; amoxicillin; amphomycin; ampicillin; ampicillin sodium; apalcillin sodium; apramycin; aspartocin; astromicin sulfate; avilamycin; avoparcin; azithromycin; azlocillin; azlocillin sodium; bacampicillin hydrochloride; bacitracin; bacitracin methylene disalicylate; bacitracin zinc; bambermycins; benzoylpas calcium; berythromycin ; betamicin sulfate; biapenem; biniramycin; biphenamine hydrochloride ; bispyrithione magsulfex; butikacin;

butirosin sulfate; capreomycin sulfate; carbadox; carbenicillin disodium; carbenicillin indanyl sodium; carbenicillin phenyl sodium; carbenicillin potassium; carumonam sodium; cefaclor; cefadroxil; cefamandole; cefamandole nafate; cefamandole sodium; cefaparole; cefatrizine; cefazaflur sodium; cefazolin; cefazolin sodium; cefbuperazone; cefdinir; cefepime; cefepime hydrochloride; cefetecol; cefixime; cefmenoxime hydrochloride; cefmetazole; cefmetazole sodium; cefonicid monosodium; cefonicid sodium; cefoperazone sodium; ceforanide; cefotaxime sodium; cefotetan; cefotetan disodium; cefotiam hydrochloride; cefoxitin; cefoxitin sodium; cefpimizole; cefpimizole sodium; cefpiramide; cefpiramide sodium; cefpirome sulfate; cefpodoxime proxetil; cefprozil; cefroxadine; cefsulodin sodium; ceftazidime; ceftibuten; ceftizoxime sodium; ceftriaxone sodium; cefuroxime; cefuroxime axetil; cefuroxime pivoxetil; cefuroxime sodium; cephacetrile sodium; cephalexin; cephalexin hydrochloride; cephaloglycin; cephaloridine; cephalothin sodium; cephapirin sodium; cephradine; cetocycline hydrochloride; cetophenicol; chloramphenicol; chloramphenicol palmitate; chloramphenicol pantothenate complex ; chloramphenicol sodium succinate; chlorhexidine phosphanilate; chloroxylenol; chlortetracycline bisulfate; chlortetracycline hydrochloride; cinoxacin; ciprofloxacin; ciprofloxacin hydrochloride; cirolemycin ; clarithromycin; clinafloxacin hydrochloride; clindamycin; clindamycin hydrochloride; clindamycin palmitate hydrochloride; clindamycin phosphate; clofazimine ; cloxacillin benzathine; cloxacillin sodium; cloxyquin; colistimethate sodium; colistin sulfate; coumermycin; coumermycin sodium; cyclacillin; cycloserine; dalfopristin; dapsone ; daptomycin; demeclocycline; demeclocycline hydrochloride; demecycline; denofungin ; diaveridine; dicloxacillin; dicloxacillin sodium; dihydrostreptomycin sulfate; dipyrithione; dirithromycin; doxycycline; doxycycline calcium ; doxycycline fosfatex; doxycycline hyclate; droxacin sodium; enoxacin; epicillin; epitetracycline hydrochloride; erythromycin; erythromycin acistrate; erythromycin estolate; erythromycin ethylsuccinate; erythromycin gluceptate; erythromycin lactobionate; erythromycin propionate; erythromycin stearate; ethambutol hydrochloride; ethionamide; fleroxacin; floxacillin; fludalanine; flumequine; fosfomycin; fosfomycin tromethamine; fumoxicillin; furazolium chloride; furazolium tartrate; fusidate sodium; fusidic acid; gentamicin sulfate; gloximonam; gramicidin; haloprogin; hetacillin; hetacillin potassium; hexedine; ibafloxacin; imipenem; isoconazole; isepamicin; isoniazid; josamycin; kanamycin sulfate; kitasamycin; levofuraltadone; levopropylcillin potassium; lexithromycin; lincomycin; lincomycin hydrochloride; lomefloxacin; lomefloxacin hydrochloride; lomefloxacin mesylate; loracarbef; mafenide;

meclocycline; meclocycline sulfosalicylate; megalomicin potassium phosphate; mequidox; meropenem; methacycline; methacycline hydrochloride; methenamine; methenamine hippurate; methenamine mandelate; methicillin sodium; metioprim; metronidazole hydrochloride; metronidazole phosphate; mezlocillin; mezlocillin sodium; minocycline; minocycline hydrochloride; mirincamycin hydrochloride ; monensin ; monensin sodium ; nafcillin sodium; nalidixate sodium; nalidixic acid; natamycin; nebramycin; neomycin palmitate; neomycin sulfate; neomycin undecylenate; netilmicin sulfate; neutramycin; nifuradene; nifuraldezone; nifuratel ; nifuratrone; nifurdazil; nifurimide; nifurpirinol; nifurquinazol; nifurthiazole; nitrocycline; nitrofurantoin; nitromide; norfloxacin; novobiocin sodium; ofloxacin; ormetoprim; oxacillin sodium; oximonam; oximonam sodium; oxolinic acid; oxytetracycline; oxytetracycline calcium; oxytetracycline hydrochloride; paldimycin; parachlorophenol; paulomycin; pefloxacin; pefloxacin mesylate; penamecillin; penicillin G benzathine; penicillin G potassium; penicillin G procaine; penicillin G sodium; penicillin V; penicillin V benzathine; penicillin V hydrabamine; penicillin V potassium; pentizidone sodium; phenyl aminosalicylate; piperacillin sodium; pirbenicillin sodium; piridicillin sodium; pirlimycin hydrochloride; pivampicillin hydrochloride; pivampicillin pamoate; pivampicillin probenate; polymyxin B sulfate; porfiromycin ; propikacin; pyrazinamide; pyrithione zinc; quindecamine acetate; quinupristin; racephenicol; ramoplanin; ranimycin; relomycin; repromicin; rifabutin; rifametane; rifamexil; rifamide; rifampin; rifapentine; rifaximin; rolitetracycline; rolitetracycline nitrate; rosaramicin; rosaramicin butyrate; rosaramicin propionate; rosaramicin sodium phosphate; rosaramicin stearate; rosoxacin; roxarsone; roxithromycin; sancycline; sanfetrinem sodium; sarmoxicillin; sarpicillin; scopafungin ; sisomicin; sisomicin sulfate; sparfloxacin; spectinomycin hydrochloride; spiramycin; stallimycin hydrochloride; steffimycin; streptomycin sulfate; streptonicozid; sulfabenz ; sulfabenzamide; sulfacetamide; sulfacetamide sodium; sulfacytine; sulfadiazine; sulfadiazine sodium; sulfadoxine; sulfalene; sulfamerazine; sulfameter; sulfamethazine; sulfamethizole; sulfamethoxazole; sulfamonomethoxine; sulfamoxole; sulfanilate zinc; sulfanitran; sulfasalazine; sulfasomizole; sulfathiazole; sulfazamet; sulfisoxazole; sulfisoxazole acetyl; sulfisoxazole diolamine; sulfomyxin; sulopenem; sultamicillin; suncillin sodium; talampicillin hydrochloride; teicoplanin; temafloxacin hydrochloride; temocillin; tetracycline; tetracycline hydrochloride ; tetracycline phosphate complex; tetroxoprim; thiamphenicol; thiphencillin potassium; ticarcillin cresyl sodium; ticarcillin disodium; ticarcillin monosodium; ticlatone; tiodonium chloride; tobramycin; tobramycin sulfate;

tosufloxacin; trimethoprim; trimethoprim sulfate; trisulfapyrimidines; troleandomycin; trospectomycin sulfate; tyrothricin; vancomycin; vancomycin hydrochloride; virginiamycin; zorbamycin.

Antiviral agents include nucleoside analogs, nonnucleoside reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors, including the following: acemannan; acyclovir; acyclovir sodium; adefovir; alovudine; alvircept sudotox; amantadine hydrochloride; aranotin; arildone; atevirdine mesylate; avridine; cidofovir; cipamfylline; cytarabine hydrochloride; delavirdine mesylate; desciclovir; didanosine; disoxaril; edoxudine; enviradene; enviroxime; famciclovir; famotine hydrochloride; fiacitabine; fialuridine; fosarilate; foscarnet sodium; fosfonet sodium; ganciclovir; ganciclovir sodium; idoxuridine; indinavir; kethoxal; lamivudine; lobucavir; memotine hydrochloride; methisazone; nelfinavir; nevirapine; penciclovir; pirodavir; ribavirin; rimantadine hydrochloride; ritonavir; saquinavir mesylate; somantadine hydrochloride; sorivudine; statolon; stavudine; tilorone hydrochloride; trifluridine; valacyclovir hydrochloride; vidarabine; vidarabine phosphate; vidarabine sodium phosphate; viroxime; zalcitabine; zidovudine; zinviroxime and integrase inhibitors.

Analgesics include acetaminophen; alfentanil hydrochloride; aminobenzoate potassium; aminobenzoate sodium; anidoxime; anileridine; anileridine hydrochloride; anilopam hydrochloride; anirolac; antipyrine; aspirin; benoxaprofen; benzydamine hydrochloride; bicifadine hydrochloride; brifentanil hydrochloride; bromadoline maleate; bromfenac sodium; buprenorphine hydrochloride; butacetin; butixirate; butorphanol; butorphanol tartrate; carbamazepine; carbaspirin calcium; carbiphene hydrochloride; carfentanil citrate; ciprefadol succinate; ciramadol; ciramadol hydrochloride; clonixeril; clonixin; codeine; codeine phosphate; codeine sulfate; conorphone hydrochloride; cyclazocine; dexoxadrol hydrochloride; dexpemedolac; dezocine; diflunisal; dihydrocodeine bitartrate; dimefadane; dipyrone; doxpicomine hydrochloride; drinidene; enadoline hydrochloride; epirizole; ergotamine tartrate; ethoxazene hydrochloride; etofenamate; eugenol; fenoprofen; fenoprofen calcium; fentanyl citrate; floctafenine; flufenisal; flunixin; flunixin meglumine; flupirtine maleate; fluproquazone; fluradoline hydrochloride; flurbiprofen ; hydromorphone hydrochloride; ibufenac; indoprofen; ketazocine; ketorfanol; ketorolac tromethamine; letimide hydrochloride; levomethadyl acetate; levomethadyl acetate hydrochloride; levonantradol hydrochloride; levorphanol tartrate; lidocaine; lofemizole hydrochloride; lofentanil oxalate; lorcinadol; lornoxicam; magnesium salicylate; mefenamic

acid; menabitan hydrochloride; meperidine hydrochloride; meptazinol hydrochloride; methadone hydrochloride; methadyl acetate; methopholine; methotrimeprazine; metkephamid acetate; mimbane hydrochloride; mirfentanil hydrochloride; molinazone; morphine sulfate; moxazocine; nabitan hydrochloride; nalbuphine hydrochloride; nalmexone hydrochloride; namoxyrate; nantradol hydrochloride; naproxen ; naproxen sodium ; naproxol; nefopam hydrochloride; nexeridine hydrochloride; noracymethadol hydrochloride; ocfentanil hydrochloride; octazamide; olvanil; oxetorone fumarate; oxycodone; oxycodone hydrochloride; oxycodone terephthalate; oxymorphone hydrochloride; pemedolac; pentamorphone; pentazocine; pentazocine hydrochloride; pentazocine lactate; phenazopyridine hydrochloride; phenyramidol hydrochloride; picenadol hydrochloride; pinadoline; pirfenidone; piroxicam olamine; pravadoline maleate; prodilidine hydrochloride; profadol hydrochloride; propiram fumarate; propoxyphene hydrochloride; propoxyphene napsylate; proxazole; proxazole citrate ; proxorphan tartrate; pyrroliphene hydrochloride; remifentanil hydrochloride; salcolex; salethamide maleate; salicylamide; salicylate meglumine; salsalate; sodium salicylate; spiradoline mesylate; sufentanil; sufentanil citrate; talmetacin; talniflumate; talosalate; tazadolene succinate; tebufelone; tetrydamine; tifurac sodium; tilidine hydrochloride; tiopinac; tonazocine mesylate; tramadol hydrochloride; trefentanil hydrochloride; trolamine; veradoline hydrochloride; verilopam hydrochloride; volazocine; xorphanol mesylate; xylazine hydrochloride; zenazocine mesylate; zomepirac sodium; zucapsaicin.

Anti-cancer compounds include, but are not limited to, the following sub-classes of compounds:

Antineoplastic agents include Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adozelesin; Adriamycin; Aldesleukin ; Altretamine; Ambomycin; Ametantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine; Busulfan; Cactinomycin; Calusterone; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; DACA (N-[2-(Dimethyl-amino)ethyl]acridine-4-carboxamide); Dactinomycin; Daunorubicin Hydrochloride; Daunomycin; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene;

Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflornithine Hydrochloride; Elsamitrucin; Enloplatin; Enpromate; Epipropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Ethiodized Oil I 131; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate; Fluorouracil; 5-FdUMP; Flurocitabine; Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Gold Au 198 ; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofosine; Interferon Alfa-2a ; Interferon Alfa-2b ; Interferon Alfa-n1; Interferon Alfa-n3; Interferon Beta- I a ; Interferon Gamma- I b; Iproplatin; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; Leuprolide Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Metoprine; Meturedepa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; Nogalamycin; Ormaplatin; Oxisuran; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; Peplomycin Sulfate; Perfosfamide; Pipobroman; Piposulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin ; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Riboprine; Rogletimide; Safingol; Safingol Hydrochloride; Semustine; Simtrazene; Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Strontium Chloride Sr 89; Sulofenur; Talisomycin; Taxane; Taxoid; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Thymitaq; Tiazofurin; Tirapazamine; Tomudex; TOP-53; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole Hydrochloride; Uracil Mustard; Uredepa; Vapreotide; Verteporfin; Vinblastine; Vinblastine Sulfate; Vincristine; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; Zorubicin Hydrochloride; 2-Chlorodeoxyadenosine; 2'-Deoxyformycin; 9-aminocamptothecin; raltitrexed; N-propargyl-5,8-dideazafolic acid; 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine; 2-chloro-2'-deoxyadenosine; anisomycin; trichostatin A; hPRL-G129R; CEP-751; linomide.

Other anti-neoplastic compounds include 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives (e.g., 10-hydroxy- camptothecin); canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; discodermolide; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; eflornithine; elemene; emitefur; epirubicin; epothilones (including A, B, desoxy B, desoxy F); epithilones; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide; etoposide 4'-phosphate (etopofos); exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant

peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; irinotecan; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide + estrogen + progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mithracin; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A + myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anticancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone + pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel and analogues thereof; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds; platinum-triamine complex; podophyllotoxin; porfimer sodium; porfiromycin; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP

inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thalidomide; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene dichloride; topotecan; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; zinostatin stimalamer.

Anti-cancer supplementary potentiating agents include tricyclic anti-depressant drugs (e.g., imipramine, desipramine, amitryptyline, clomipramine, trimipramine, doxepin, nortriptyline, protriptyline, amoxapine and maprotiline); non-tricyclic anti-depressant drugs (e.g., sertraline, trazodone and citalopram); Ca^{++} antagonists (e.g., verapamil, nifedipine, nitrendipine and caroverine); calmodulin inhibitors (e.g., prenylamine, trifluoroperazine and clomipramine); Amphotericin B; triparanol analogues (e.g., tamoxifen); antiarrhythmic drugs (e.g., quinidine); antihypertensive drugs (e.g., reserpine); thiol depleters (e.g., buthionine and sulfoximine) and multiple drug resistance reducing agents such as Cremaphor EL. The compounds of the invention also can be administered with cytokines such as granulocyte colony stimulating factor (G-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF).

Antiproliferative agents include Piritrexim Isethionate.

Antiprostatic hypertrophy agents include Sitogluside.

Benign prostatic hyperplasia therapy agents include Tamsulosin Hydrochloride.

Prostate growth inhibitor agents include Pentomone.

Radioactive agents include Fibrinogen I 125 ; Fludeoxyglucose F 18; Fluorodopa F 18 ; Insulin I 125; Insulin I 131; Iobenguane I 123; Iodipamide Sodium I 131; Iodoantipyrine I 131; Iodocholesterol I 131; Iodohippurate Sodium I 123; Iodohippurate Sodium I 125; Iodohippurate Sodium I 131; Iodopyracet I 125; Iodopyracet I 131; Iofetamine Hydrochloride I 123; Iomethin I 125; Iomethin I 131; Iothalamate Sodium I 125; Iothalamate Sodium I 131; Iotyrosine I 131; Liothyronine I 125; Liothyronine I 131; Merisoprol Acetate Hg 197; Merisoprol Acetate Hg 203; Merisoprol Hg 197; Selenomethionine Se 75; Technetium Tc 99m Antimony Trisulfide Colloid; Technetium Tc 99m Bicisate; Technetium Tc 99m Disofenin; Technetium Tc 99m Etidronate; Technetium Tc 99m Exametazime; Technetium Tc 99m Furifosmin; Technetium Tc 99m Gluceptate; Technetium Tc 99m Lidofenin; Technetium Tc 99m Mebrofenin; Technetium Tc 99m Medronate; Technetium Tc 99m Medronate Disodium; Technetium Tc 99m Mertiatide; Technetium Tc 99m Oxidronate; Technetium Tc 99m Pentetate; Technetium Tc 99m Pentetate Calcium Trisodium; Technetium Tc 99m Sestamibi; Technetium Tc 99m Siboroxime; Technetium Tc 99m Succimer; Technetium Tc 99m Sulfur Colloid; Technetium Tc 99m Teboroxime; Technetium Tc 99m Tetrofosmin; Technetium Tc 99m Tiatide; Thyroxine I 125; Thyroxine I 131; Tolpovidone I 131; Triolein I 125; Triolein I 131.

The reduction of tissue damage by dehydroascorbic acid also enables improvements in organ transplantation perfusion fluids used in the treatment, storage and transport of organs to be transplanted. Thus the invention also provides medical products useful in organ transplantation. In particular, the invention provides organ perfusion fluids containing dehydroascorbic acid, as well as organs perfused with such perfusion fluids. One of ordinary skill in the art is familiar with standard organ perfusion fluids, including University of Wisconsin solution, Euro-Collins solution, BT01 solution, Ringer's lactate solution and normal saline solution. Other non-dehydroascorbic acid organ perfusion agents which can be added to the foregoing perfusion solutions include calcium entry blockers (e.g. lidoflazine), cytoprotectors (e.g., natriuretic factor, PGI2, trimetazidine), free radical chelating agents and scavengers (e.g., allopurinol, mannitol, glutathione), and substrates for the mitochondrial respiratory chain (e.g., aspartate, glutamate).

Also provided are methods for *ex vivo* preservation of a tissue or organ. The methods include contacting the tissue or organ *ex vivo* with an amount of an organ perfusion fluid

containing a buffered dehydroascorbic acid composition as described herein effective to increase the concentration of ascorbic acid in the tissue or organ.

Kits containing dehydroascorbic acid compositions, preferably buffered, in effective amounts also are provided. The kits contain one or more containers with the dehydroascorbic acid compositions of the invention along with instructions for mixing, diluting and/or administering the dehydroascorbic acid compositions in effective amounts. The kits also can include other containers with one or more buffers, solvents, surfactants, preservatives and/or diluents, as well as containers for mixing, diluting, and/or administering the compositions to a subject in need of such treatment.

The dehydroascorbic acid compositions in the kit may be provided as liquid solutions, or preferably, as dried powders to be reconstituted prior to administration. When the dehydroascorbic acid compositions provided is a dry powder, the powder may be reconstituted by the addition of a suitable solvent, which also may be provided. Liquid forms of the dehydroascorbic acid compositions may be concentrated (for dilution prior to administration) or ready to administer to a subject.

The preparations, formulations and compositions containing dehydroascorbic acid may be encapsulated by liposomes or other microparticles, according to standard procedures for preparation of such compositions.

The compositions of the invention are administered in effective amounts. An "effective amount" is that amount of a buffered dehydroascorbic acid composition that alone, or together with further doses, produces the desired response, e.g. increases ascorbic acid in a desired tissue. In the case of treating a particular disease or condition the desired response is inhibiting the progression of the disease or condition. This may involve only slowing the progression of the disease temporarily, although more preferably, it involves halting the progression of the disease permanently. This can be monitored by routine diagnostic methods known to one of ordinary skill in the art for any particular disease. The desired response to treatment of the disease or condition also can be delaying the onset or even preventing the onset of the disease or condition.

Such amounts will depend, of course, on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size and weight, the duration of the treatment, the nature of concurrent therapy (if any), the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can

be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the dehydroascorbic acid composition (alone or in combination with other therapeutic agents) be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

The pharmaceutical compositions used in the foregoing methods preferably are sterile and contain an effective amount of dehydroascorbic acid for producing the desired response in a unit of weight or volume suitable for administration to a patient. The response can, for example, be measured by determining the physiological effects of the dehydroascorbic acid composition, such as the decrease of disease symptoms following administration of the dehydroascorbic acid composition,. Other assays will be known to one of ordinary skill in the art and can be employed for measuring the level of the response.

The doses of buffered dehydroascorbic acid compositions administered to a subject can be chosen in accordance with different parameters, in particular in accordance with the mode of administration used and the state of the subject. Other factors include the desired period of treatment. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits.

Various modes of administration will be known to one of ordinary skill in the art which effectively deliver the buffered dehydroascorbic acid composition to a desired tissue, cell or bodily fluid. Preferred methods for administering the buffered dehydroascorbic acid of the invention include topical, intravenous, oral, intracavity, intrathecal, intrasynovial, buccal, sublingual, intranasal, transdermal, intravitreal, subcutaneous, intramuscular, intrarectal lavage and intradermal administration. Although these are preferred embodiments, the invention is not limited by the particular modes of administration disclosed herein. Standard references in the art (e.g., *Remington's Pharmaceutical Sciences*, 18th edition, 1990) provide modes of administration and formulations for delivery of various pharmaceutical preparations and formulations in pharmaceutical carriers. Other protocols which are useful for the administration of dehydroascorbic acid compositions will be known to one of ordinary skill in the art, in which the dose amount, schedule of administration, sites of administration, mode of administration (e.g., intra-organ) and the like vary from those presented herein.

Administration of buffered dehydroascorbic acid compositions to mammals other than humans, e.g. for testing purposes or veterinary therapeutic purposes, is carried out under substantially the same conditions as described above. It will be understood by one of ordinary skill in the art that this invention is applicable to both human and animal diseases which can be treated by dehydroascorbic acid. Thus this invention is intended to be used in husbandry and veterinary medicine as well as in human therapeutics. As used herein, therefore, "subject" includes humans, non-human primates, cows, pigs, horses, sheep, dogs, cats, mice, rats, etc.

When administered, the pharmaceutical preparations of the invention are applied in pharmaceutically-acceptable amounts and in pharmaceutically-acceptable compositions. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. Such preparations may routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts. Preferred components of the composition are described above in conjunction with the description of the buffered dehydroascorbic acid compositions of the invention.

A dehydroascorbic acid composition may be combined, if desired, with a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with dehydroascorbic acid, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

The pharmaceutical compositions may contain suitable buffering agents, as described above, including: acetate, phosphate, citrate, glycine, borate, carbonate, bicarbonate, hydroxide (and other bases) and pharmaceutically acceptable salts of the foregoing compounds. Particularly preferred buffer molecules include sodium bicarbonate and sodium acetate.

The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal. Preferably the preservative is EDTA or sodium benzoate.

The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well-known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, lozenges, each containing a predetermined amount of the active compound. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir, emulsion, or mouthwash.

Compositions suitable for parenteral administration conveniently comprise a sterile aqueous buffered dehydroascorbic acid composition. This preparation may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also may be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid may be used in the preparation of injectables. Carrier formulation suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA.

Examples

Example 1: Assessment of toxicity of buffered DHA compositions

This study was designed to assess the toxicity of dehydroascorbic acid (DHA) when administered via a single intravenous (bolus) injection to Sprague-Dawley CD® rats at dose levels of 2, 20, 100, 300 and 500 mg/kg followed by a 7-day observation period. Control animals were administered ascorbic acid (AA) at a dose level of 500 mg/kg via a single intravenous (bolus) injection followed by a 7-day observation period.

Materials and methods

Good laboratory practices

This study was conducted in compliance with Part 58 of 21 CFR (FDA Good Laboratory Practice Standards).

Animal welfare compliance

This study complied with all appropriate parts of the Animal Welfare Act Regulations: 9 CFR Parts 1 and 2 Final Rules, Federal Register, Volume 54, No. 168, August 31, 1989, pp. 36112-36163 effective October 30, 1989 and 9 CFR Part 3 Animal Welfare Standards; Final Rule, Federal Register, Volume 56, No. 32, February 15, 1991, pp. 6426-6505 effective March 18, 1991.

Facilities management/animal husbandry

Currently acceptable practices of good animal husbandry were followed e.g., *Guide for the Care and Use of Laboratory Animals*; National Academy Press, 1996.

Experimental design

Animals were dosed with the dehydroascorbic acid and ascorbic acid compositions according to the following dosing schedule:

		Dose[a]			Number of Animals			
Group	Test Article	Dose	Volume	Conc[a]	Total		Necropsy (Day 8)	
		(mg/kg)	(mL/kg)	(mg/mL)	M	F	M	F
1	DHA	2	2	1	1	1	1	1
2	DHA	20	2	10	1	1	1	1
3	DHA	100	2	50	1	1	1	1
4	DHA	300	2	150	2	2	2	2
5	DHA	500	2	250	2	2	2	2
6	AA	500	2	250	2	2	2	2

[a]Dose solution diluted as necessary in normal saline.

DHA = Dehydroascorbic acid, AA = Ascorbic acid, Conc = Concentration, M = Male, F = Female

mg/kg = milligrams of test article per kilogram of body weight.

Test animals

The animals (Albino Rats (Outbred) VAF/Plus®, Sprague-Dawley - derived (CD®), Crl:CD® (SD)IGS BR) were obtained from Charles River Laboratories (Kingston, New York). These animals were chosen because the rat is an animal model commonly utilized in toxicity studies. In addition, a historical data base is available for comparative evaluation.

In total, 24 animals were received (12 males, 12 females). Of these, 18 total (9 males, 9 females) were used in the testing. Females were nulliparous and non-pregnant. The age of the animals at receipt was 29 days old. The age at initiation of dosing was 43 days old.

At initiation of dosing, the weight of the animals (in grams) was as follows

	Mean	**Range**
Male:	210	190-231
Female:	143	136-150

Individual weights of animals placed on test were within ±20% of the mean weight for each sex.

Animals were acclimated for 2 weeks. All animals were examined during the acclimation period to confirm suitability for study. Animals considered suitable for the study on the basis of pretest physical examinations and body weight data were distributed into 3 groups of 1 animal per sex (Groups 1-3) and 3 groups of 2 animals per sex (Groups 4-6). Animals were distributed into groups using a computer generated random numbers table.

Each rat was identified with a metal ear tag bearing its assigned animal number. The assigned animal number plus the study number comprised the unique animal number for each animal. In addition, each cage was provided with a cage card which was color-coded for dose level identification and contained study number and animal number information.

Animals were monitored by the technical staff for any conditions requiring possible veterinary care.

Animals were doubly housed in elevated, stainless steel, wire mesh cages during the first week of the acclimation period and individually housed thereafter. The animal feed, Certified Rodent Diet, No. 5002; (Meal) (PMI Nutrition International, St. Louis, Missouri) was available without restriction. Fresh feed was presented weekly. Water was available without restriction via an automated watering system.

A twelve hour light/dark cycle controlled via an automatic timer was provided. Temperature was monitored and recorded twice daily and maintained within the specified range to the maximum extent possible. The desired range was 18 to 26°C. The actual temperature range was 19 to 23°C. Relative humidity was monitored and recorded once daily and maintained within the specified range to the maximum extent possible. The desired range was 30 to 70% relative humidity. The actual range was 36 to 74%.

Preparation of dosing solutions

Appropriate amounts of the test and control articles were mixed with the vehicle to achieve the desired concentrations. The method of preparation was as follows. The Sodium Acetate Buffer was prepared by dissolving 8.20 grams (0.1 Mole) of anhydrous sodium acetate in 30 ml distilled water and adjusting the pH to 5.5 with 1.0 Normal acetic acid. The resulting solution was diluted to 100 ml with distilled water. This buffer was stored in the refrigerator and was stable for at least one month. A sodium bicarbonate solution was

prepared by dissolving an equimolar amount of sodium bicarbonate (molecular weight 84.11) to the amount of DHA in the volume of the final solution of DHA. This solution was heated to approximately 40°C for dissolution and was prepared fresh for each DHA preparation. The dosing solution of DHA (250 mg/ml) was prepared by weighing the dry DHA (molecular weight 174.11) into a clean dry container, then adding 85% of the sodium bicarbonate solution made as described above and stirring at room temperature for 30 minutes. The remaining 15% volume of sodium acetate buffer prepared as described above was added to the DHA solution and the solution was stored at room temperature in brown glass or foil-wrapped vials.

The pH of the dosing solution was checked and adjusted to 5.0 ± 0.2 using the above solutions. Dosing solutions were prepared under aseptic conditions and filtered through a 0.22 micron filter prior to dose administration. The dosing solution was administered within 8 hours of preparation. Control animals received control article formulated in the same vehicle and at the same concentration as the test article.

The test and control articles were administered by intravenous injection into the tail vein using a needle and syringe of appropriate size. Doses were calculated using the most recent body weights available.

The test article was administered as a single dose, followed by a 7 day observation period. The dose levels administered were as follows:

Group 1	-	2 mg/kg DHA
Group 2	-	20 mg/kg DHA
Group 3	-	100 mg/kg DHA
Group 4	-	300 mg/kg DHA
Group 5	-	500 mg/kg DHA
Group 6	-	500 mg/kg AA

The dose volume administered was 2 mL/kg for all groups.

The study was designed to establish a maximum-tolerated dose for the test article and was based upon a survey of published literature on the *in vivo* effects of the test article formulated in other vehicles.

Experimental evaluations

Viability checks

Animals were observed in their cages twice daily for mortality and general condition.

Clinical signs (cage-side)

Observations for signs of toxic or pharmacologic effects were made once daily for each animal. Unusual signs were recorded. These observations were made concurrently with one of the viability checks.

Dose observations

Observations for signs of toxic or pharmacologic effects were made during and just after dosing; all unusual signs were recorded.

Physical examinations

Animals were removed from their cages and examined twice pretest and daily during the study period. Examinations included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration and palpation for tissue masses.

Body weight

Animals were removed from their cages and weighed twice pretest, on Day 7 and fasted body weights were obtained prior to necropsy.

Food consumption

Feed was available without restriction 7 days/week. Animals were presented with full feeders weighing 570 grams (includes weight of feed, jar and lid). After 6 days, feeders were reweighed and the resulting weight was subtracted from the full feeder weight to obtain the grams consumed per animal over the 6-day period. Food consumption was measured (weighed) weekly, beginning one week prior to treatment.

Calculation

grams of food consumed/kilogram of body weight/day (g/kg/day) =

$$\frac{\text{grams of food consumed}}{\text{body weight (kg)}^{a}} \div 6 \text{ days}$$

[a]The average of the current and previous weight was used.

Postmortem analysis

Euthanasia was performed by exsanguination following carbon dioxide inhalation on test day 8. Complete macroscopic postmortem examinations were performed on all animals sacrificed at the scheduled sacrifice interval immediately after death. The macroscopic postmortem examination included an external examination, including identification of all clinically-recorded lesions, as well as a detailed internal examination. Animals were fasted prior to the scheduled sacrifice.

Results

All animals survived the administration of the test and control dosing solutions. Animals receiving 500 mg/kg DHA and 500 mg/kg AA vocalized during dosing, suggesting that the test articles were irritating. Clinical signs observed immediately postdose in animals receiving 300 and 500 mg/kg DHA were lethargy, rapid breathing, and loss of color (paleness). These effects were not observed in the animals treated with AA. All the animals recovered from these effects in a short time (1-3 minutes). Thereafter, all animals were within normal limits during the seven-day observation period. There were no test article related effects on body weight and food consumption values were considered comparable between treatment groups.

There were no findings of macroscopic pathology related to treatment with either DHA or AA. There was an observation of kidney dilation in a male receiving 2 mg/kg DHA; this was considered to be an incidental finding.

Conclusion

Administration of 300 and 500 mg/kg DHA intravenously to Sprague-Dawley rats resulted in an immediate response characterized by vocalization, lethargy, rapid breathing,

and paleness. Vocalization was also observed in the animals receiving AA. A maximum-tolerated dose was not determined due to the transient nature of the clinical signs.

Example 2: Assessment of maximum tolerated dose of buffered DHA compositions

This study was designed to identify a maximum tolerated dose of dehydroascorbic acid (DHA) when administered via a single intravenous (bolus) injection to Sprague-Dawley CD® rats at dose levels of 750, 1000, 1250, 1500, 1750 and 2000 mg/kg followed by a 7-day observation period. Control animals were administered ascorbic acid (AA) at a dose level of 1750 mg/kg via a single intravenous (bolus) injection followed by a 7-day observation period.

Materials and methods

The materials and methods used were generally the same as Example 1 above. Any differences in material or methods are noted below.

Animals were dosed with the dehydroascorbic acid and ascorbic acid compositions according to the following dosing schedule:

		Dose[a]			Number of Animals			
Group	Test Article	Dose	Volume	Conc[a]	Total		Necropsy (Day 8)	
		(mg/kg)	(mL/kg)	(mg/mL)	M	F	M	F
1	DHA	750	8	93.75	2	2	2	2
2	DHA	1000	8	125	2	2	2	2
3	DHA	1250	8	156.25	2	2	2	1[b]
4	DHA	1500	8	187.5	2	2	1[b]	1[b]
5	DHA	1750	8	218.75	2	2	0[b]	0[b]
6	DHA	2000	8	250	2	2	0[b]	0[b]
7	AA	1750	8	218.75	2	2	0[b]	0[b]

[a] Dose solution diluted as necessary in normal saline.

[b] Remaining animals were found dead on Day 0 (the day of the dose).

DHA = Dehydroascorbic acid, AA = Ascorbic acid, Conc = Concentration, M = Male, F = Female

mg/kg = milligrams of test article per kilogram of body weight.

Test animals

The animals (Albino Rats (Outbred) VAF/Plus®, Sprague-Dawley - derived (CD®), Crl:CD® (SD)IGS BR) were obtained from Charles River Laboratories (Kingston, New York). These animals were chosen because the rat is an animal model commonly utilized in toxicity studies. In addition, a historical data base is available for comparative evaluation.

In total, 44 animals were received (22 males, 22 females). Of these, 28 total (14 males, 14 females) were used in the testing. Females were nulliparous and non-pregnant. The age of the animals at receipt was 4 weeks old. The age at initiation of dosing was 6 weeks old.

At initiation of dosing, the weight of the animals (in grams) was as follows

	Mean	**Range**
Male:	208	190-227
Female:	165	153-183

Individual weights of animals placed on test were within ±20% of the mean weight for each sex.

Ambient room temperature was monitored and recorded twice daily and maintained within the specified range to the maximum extent possible. The desired range was 18 to 26°C. The actual temperature range was 21 to 23°C. Relative humidity was monitored and recorded once daily and maintained within the specified range to the maximum extent possible. The desired range was 30 to 70% relative humidity. The actual range was 28 to 58%.

Preparation of dosing solutions

The dosing solution of DHA (500 mg/ml) was prepared by weighing the dry DHA (molecular weight 174.11) into a clean dry container, then adding 85% of the sodium bicarbonate solution made as described above and stirring at room temperature for 30 minutes. The sodium acetate buffer prepared as described above was added to bring the

DHA solution to final volume and the solution was stored at room temperature in brown glass or foil-wrapped vials. The dosing solutions of Groups 1-3 were prepared by diluting Group 4 solution to the appropriate concentration with sodium bicarbonate solution and the pH adjusted with sodium bicarbonate or sodium acetate solutions.

Test article administration

The test and control articles were administered by intravenous injection into the tail vein using a needle and syringe of appropriate size. Treatment was administered in a multiple step process. On the afternoon of Day 0, Groups 1-4 were administered test article at dose levels of 750, 1000, 1250 and 1500 mg/kg DHA, respectively. These dose levels failed to reveal 100% lethality. Therefore, on the following day animals in Groups 5 and 6 were administered test article at dose levels of 1750 and 2000 mg/kg DHA in the morning. Lethality of 100% was found at 1750 and 2000 mg/kg DHA. A dose of AA was administered as a reference control at the lowest dose of DHA that resulted in 100% lethality (1750 mg/kg). The dose volume for each group was 8 mL/kg.

Postmortem

Euthanasia was performed by exsanguination following carbon dioxide inhalation on test day 7.

Results

One animal receiving 1250 mg/kg DHA died immediately postdose and 2 animals in the 1500 mg/kg DHA group died immediately postdose. All animals in the 1750 and 2000 mg/kg DHA groups and the 1750 AA group died immediately postdose. All other animals survived to their scheduled termination date.

Clinical signs observed immediately postdose in animals at all dose levels of DHA were lethargy and labored or rapid breathing. Some animals were prostrate immediately postdose, then regained their normal posture. Some animals had bruised tails which later became necrotic. Thereafter, all surviving animals were within normal limits during the seven-day observation period. There were no test article related effects on body weight or food consumption.

Conclusion

Intravenous administration of DHA at doses ranging from 750-1500 mg/kg to Sprague-Dawley rats resulted in an immediate response characterized by lethargy, labored or rapid breathing, and a prostate posture. One of four animals receiving 1250 mg/kg DHA and two of four animals receiving 1500 mg/kg DHA died immediately postdose. At doses of 1750 and 2000 mg/kg DHA and 1750 mg/kg AA there was 100% lethality immediately postdose. The maximum tolerated dose of DHA was determined to be 1000 mg/kg. The LD50 of DHA was determined to be approximately 1500 mg/kg.

Example 3: Preparation of dehydroascorbic acid formulations

Formulations of DHA useful in various methods of administration were prepared as follows. As shown in other examples, these preparations can be scaled up or down in volume as required.

A. Preparation of an intravenous formulation of dehydroascorbic acid

1 M sodium acetate buffer

Per liter:

1. Dissolve 82.0 grams (1 mole) of anhydrous sodium acetate (catalog #S1429, Sigma Chemical Co., St. Louis, MO, or equivalent) in 300 mL distilled water.
2. Adjust to pH 5.5 with 1.0 N acetic acid (57.5 mL glacial acetic acid/L).
3. Dilute to 1 L with distilled water.
4. Store at 4 C for 1 month.

Note: ~200 mL of 1 0 N acetic acid is required. After adjustment to 1 L, the final solution pH is ~5.4.

1.44 M sodium bicarbonate

Per liter:

Dissolve 121.1 grams of sodium bicarbonate (Sigma catalog #S1554 or equivalent) in 1 liter of water.

Note: This solution needs to be heated to ~40 C for dissolution.

Note: This solution must be prepared fresh for each DHA preparation.

250 mg/ml (1.44 M) dehydroascorbic acid

Per liter:

1. Weigh 250 g of dry DHA (catalog # 30790, Fluka, Milwaukee, WI or equivalent).
2. Add 850 ml of 1.44 M sodium bicarbonate.
3. Dissolve by swirling or magnetic mixing for 30 minutes at room temperature.
4. Add 150 ml of 1 M sodium acetate.
5. Check pH and adjust to 5.0 with 1.44 M sodium bicarbonate or 1.0 N acetic acid.

B. Preparation of a PEG 400-containing formulation of dehydroascorbic acid

This formulation is suitable for topical use.

1.05 M sodium acetate buffer

Per liter:

1 . Dissolve 86.1 grams (1.05 mole) of anhydrous sodium acetate (Sigma catalog #S1429 or equivalent) in 300 ml, distilled water.

2. Adjust to pH 5.5 with 1.0 N acetic acid (57.5 mL glacial acetic acid/L).
3. Dilute to 1 L with distilled water.
4. Store at 4 C for 1 month.

Note: ~200 mL of 1 0 N acetic acid is required. After adjustment to 1 L, the final solution pH is ~5.4.

1.51 M sodium bicarbonate

Per liter:

Dissolve 127.2 grams of sodium bicarbonate (Sigma catalog #S1554 or equivalent) in 1 liter of water.

Note: This solution needs to be heated to ~40 C for dissolution.

Note: This solution must be prepared fresh for each DHA preparation.

250 mg/ml (1.44 M) dehydroascorbic acid

Per liter:

1. Weigh 250 g of dry DHA (Fluka catalog # 30790 or equivalent).
2. Add 808 ml of 1.51 M sodium bicarbonate.
3. Dissolve by swirling or magnetic mixing for 30 minutes at room temperature.
4. Add 142 ml of 1.05 M sodium acetate.

5. Add 50 ml of PEG 400 liquid (catalog #U216, Baker grade, J.T. Baker Chemicals, Phillipsburg, NJ).
6. Check pH and adjust to 5.0 with 1.51 M sodium bicarbonate or 1.0 N acetic acid.

Example 4: Effect of Dehydroascorbic Acid on Oral Mucositis

The acute radiation model in hamsters has proven to be an accurate, efficient and cost-effective technique to evaluate anti-mucositis compounds. The acute model has little systemic toxicity, resulting in fewer animal deaths, permitting the use of smaller groups (N=8) in for initial efficacy studies. It has also been used to study specific mechanistic elements in the pathogenesis of mucositis. In this study, an acute radiation dose of 35 Gy on day 0 was administered in order to produce severe mucositis around day 15.

The objective of this investigation was to evaluate the effect of DHA on the development and resolution of oral mucositis induced by acute radiation and to compare the effect of dose on the severity of ulcerative mucositis.

The compound dehydroascorbic acid (DHA) is a derivative of vitamin C. DHA has antioxidant characteristics and may inhibit free radical formation. If DHA does act as a scavenger of free radicals, it may have an impact on at least one of the important components in the pathogenesis of mucositis. Consequently, the study investigated the efficacy of DHA in the prevention and resolution of acute oral mucositis in an animal model.

To evaluate the value of DHA in the prevention and/or treatment of mucositis, animals began treatment on day -1, the day prior to irradiation (day 0 = day of irradiation). Animals were randomized to receive one of the following: (i) DHA in dimethylsulfoxide (DMSO), (ii) DMSO vehicle control, (iii) DHA in aqueous solution, (iv) aqueous vehicle control, or (v) Ascorbic Acid in aqueous solution. Hamsters continued to receive treatment daily from day -1 through day 21.

Forty-eight (48) hamsters were used. The hamsters were randomized into six (6) equally sized groups on day -1. Each group was assigned a different treatment as follows:
Group 1: topical DHA (250 mg/ml) in DMSO, 0.2 ml, tid, day -1 to day 21;
Group 2: topical DMSO vehicle control, 0.2 ml, tid, day -1 to day 21;
Group 3: topical DHA (25 mg/ml), in aqueous solution, 0.2 ml, tid, day -1 to day 21;
Group 4: topical DHA (250 mg/ml), in aqueous solution, 0.2 ml, tid, day -1 to day 21;
Group 5: topical aqueous solution (vehicle control), 0.2 ml, tid, day -1 to day 21;

Group 6: topical ascorbic acid (250 mg/ml) in aqueous solution, 0.2 ml, tid, day -1 to day 21.

The main protocol events were:

i) Every day for the period of the study (Day -1 to Day 28), each animal was weighed and its behavior and survival recorded.

ii) Each animal was irradiated on Day 0.

iii) Animals were dosed three times daily from day -1 to day 21.

iv) Starting on Day 6 and continuing every second day thereafter (Days 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 28), each animal was photographed and evaluated for mucositis scoring.

Material and Methods

Study Locations

The study was performed at The Massachusetts College of Pharmacy and Allied Health, Boston, MA, USA. This study was approved by the Animal Use Committee of the Massachusetts College of Pharmacy and Allied Health prior to study initiation. Animals were irradiated at The Dana Farber Cancer Institute, Boston, MA, USA.

Animals

Male Golden Syrian hamsters (Charles River Laboratories or Harlan Sprague Dawley), aged 5 to 6 weeks, with body weight approximately 90 g at study commencement, were used. Animals were individually numbered using an ear punch and housed in small groups of approximately 6 animals per cage. Animals were acclimatized for at least one week prior to study commencement. During this period, the animals were observed daily in order to reject animals that were in poor condition.

Housing

The study was performed in animal rooms provided with filtered air at a temperature of 70 ± 5°F and 50 ± 20% relative humidity. Animal rooms were set to maintain a minimum of 12 to 15 air changes per hour. The room was on an automatic timer for a light - dark cycle of 12 hours on and 12 hours off with no twilight. Hardwood shavings (Aspen) from Northeast Bedding Supply were used. Bedding was packaged in vacuum-packaged bags and irradiated prior to use. Bedding was changed a minimum of once per week.

Cages, tops, bottles, etc. were washed with a commercial detergent and allowed to air dry. Prior to use, these items were wrapped and autoclaved. Cage change was done in a flow hood. A commercial disinfectant was used to disinfect surfaces and materials introduced into the hood.

Floors were swept daily and mopped a minimum of twice weekly with a commercial detergent. Walls and cage racks were sponged a minimum of once per month with a dilute bleach solution.

A cage card or label with the appropriate information necessary to identify the study, dose, animal number and treatment group marked all cages. The temperature and relative humidity were recorded during the study, and the records retained.

Diet

Animals were fed with a standard hamster chow and water *ad libitum.*

Animal Randomization and Allocations.

Hamsters were randomly and prospectively divided into six (6) treatment groups prior to irradiation. Each animal was identified by an ear punch corresponding to an individual number. For more consistent identification, ear punch numbering was used rather than tagging, since tags may become dislodged during the course of the study. A cage card identified each cage or label marked with the study number, treatment group number and animal numbers.

Mucositis Induction

Mucositis was induced using an acute radiation protocol. A single dose of radiation (35 Gy/dose) was administered to all animals on Day 0. Radiation was generated with a 250 kilovolt potential (15-ma) source at a focal distance of 50 cm, hardened with a 0.35 mm Cu filtration system. Irradiation targeted the left buccal pouch mucosa at a rate of 121.5 cGy/minute. Prior to irradiation, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (80 mg/kg). The left buccal pouch was everted, fixed and isolated using a lead shield.

Topical Drug Dosing

Each group was treated with the contents of a single 200 ml bottle of the fully formulated compound. The aqueous buffered formulation of dehydroascorbic acid was prepared as described in Example 3B. For each dosing, a volume of 2 ml of compound was removed from each of the sealed bottles using a sterile tuberculin syringe fitted with a sterile needle. The solution was transferred to a needleless tuberculin syringe. Using the needleless syringe, a volume of 0.2 ml of the test compound was inserted into the base of the left cheek pouch of each animal into which the test material was deposited. When not in use, all bottles were stored in a refrigerator at 4 C.

Mucositis Evaluation

The parameters measured were the mucositis score, weight change and survival. For the evaluation of mucositis, the animals were anesthetized with inhalation anesthetics, and the left pouch everted and photographed with a Yashica Dental Eye camera. Mucositis was scored visually by comparison to a validated photographic scale, ranging from 0 for normal, to 5 for severe ulceration. In descriptive terms, this scale is defined as follows:

Score	Description
0	Pouch completely healthy. No erythema or vasodilation.
1	Light to severe erythema and vasodilation. No erosion of mucosa.
2	Severe erythema and vasodilation. Erosion of superficial aspects of mucosa leaving denuded areas. Decreased stippling of mucosa.
3	Formation of off-white ulcers in one or more places. Ulcers may have a yellow/gray appearance due to pseudomembrane formation. Cumulative size of ulcers should equal about ¼ of the pouch. Severe erythema and vasodilation.
4	Cumulative size of ulcers should equal about ½ of the pouch. Loss of pliability. Severe erythema and vasodilation.
5	Virtually all of pouch is ulcerated. Loss of pliability (pouch can only partially be extracted from mouth).

A score of 1-2 is considered to represent a mild stage of the disease, whereas a score of 3-5 is considered to indicate moderate to severe mucositis. Following visual scoring, a photograph was taken of each animal's mucosa using a standardized technique. At the

conclusion of the experiment all film was developed and the photographs randomly numbered. At least two independent trained observers graded the photographs in blinded fashion using the above-described scale (blinded scoring).

Assessment of Results

Statistical differences between treatment groups were determined using Student's *t*-test, Mann Whitney U test and chi-square analysis with a critical value of 0.05.

Results and Discussion

Survival

Four animal deaths occurred during this study. All deaths occurred on day 0 and were due to an overdose of anesthesia. The possibility of anesthesia death was a consideration in the design of this study. The aqueous buffer control group was reduced to an N of 6 while the groups treated with 250 mg/ml DHA in aqueous buffer and 250 mg/ml ascorbic acid in aqueous buffer were reduced to an N of 7. These numbers were satisfactory for the analyses performed.

Weight

Percent weight change. Animals were weighed daily, the percent weight change from day 0 was calculated, and group means and standard errors of the mean (SEM) calculated for each day. Animals in the aqueous buffer control group gained approximately 35% above their starting weight during the course of this study. By contrast, the DMSO control group gained only 29% of their starting weight, a lag in weight gain occurring on day 10 and continuing for the rest of the study. An area under the curve (AUC) analysis indicated that the difference in weight gain between the aqueous buffer control and the DMSO control was significant ($p=0.009$).

The group treated with 250 mg/ml DHA in DMSO showed a lag in weight gain similar to that observed in the DMSO control group. It appears that DMSO itself causes weight loss when administered topically to the mouth. It seems likely that DMSO causes a loss of appetite, probably due to nausea. This property of DMSO may be a significant impediment to its clinical applicability.

Of the animals treated with aqueous solutions, those receiving 25 mg/ml of DHA showed a significant reduction in weight gain by AUC analysis ($p=0.039$) compared to the

aqueous control. The groups treated with 250 mg/ml DHA or 250 mg/ml ascorbic acid gained weight at a rate that was statistically the same as the control group.

Mucositis

Mean group mucositis scores were obtained for each treatment group.

The Aqueous Buffer Control Group.

The course of mucositis in the aqueous buffer control group was characteristic of the course of mucositis typically observed. Early mucositis began by day 10-12, lesions appeared in some animals by day 14 and peak mucositis occurred on day 16. From day 16 to the end of the study, the lesions healed and were nearly resolved by day 28.

The scores for the aqueous buffer control group were typical for the acute radiation model. The peak mucositis score was 3.2. While there was a significant level of ulcerative mucositis, animals in the control spent 45.8% of the study with ulcerations as indicated by scores of 3 or more. The study had the ability to demonstrate both efficacy and worsening as a result of treatment with the experimental compounds.

The DMSO Control Group.

The response of this group was temporally different form the aqueous buffer control group; the onset of mucositis was earlier, starting on day 6 and peaking on day 14. While the aqueous buffer control group resolved mucositis slowly over the second half of the study, ulcerative lesions in the DMSO control group resolved more rapidly, reaching a mean score of 1.2 by day 22. Hamsters in the DMSO control group spent 24% of the study days with a score of 3 or more, a significant reduction in the severity of mucositis ($p<0.001$). This unanticipated result suggests that DMSO itself may show efficacy in treating oral mucositis. The improvement occurred predominately in the healing phase of the study (days 18-24) as determined both by rank sum analysis (Table 1) and by chi-square analysis of duration of ulceration (Table 2).

Table 1

Mann Whitney Rank Sum Test of Daily Mucositis Scores (P Values)
Significant Efficacy is Underlined, Significant worsening in *Italics*

	Day											
Group Comparison	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**
Aqueous Buffer Control v. DMSO Control	0.148	*<0.001*	*0.001*	*0.034*	*0.023*	0.852	0.006	0.002	<0.001	0.017	0.051	0.084
DMSO Control v. 250 mg/ml DHA in DMSO	0.472	0.955	1.000	*0.002*	*0.002*	*0.016*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.006*	*0.002*
Aqueous Buffer Control v. 25 mg/ml DHA in Aqueous Buffer	0.053	*0.027*	0.470	0.016	0.183	0.294	0.270	0.183	0.006	0.017	<0.001	0.084
Aqueous Buffer Control v. 250 mg/ml DHA in Aqueous Buffer	0.453	*0.009*	0.104	0.013	0.156	0.439	0.979	0.204	0.568	1.000	0.197	0.588
Aqueous Buffer Control v. 250 mg/ml Ascorbic Acid in Aqueous Buffer	*0.047*	0.897	0.622	0.163	0.737	0.179	0.066	0.114	0.022	0.135	0.012	0.235

Table 2

Chi Square analysis of Daily Mucositis Scores ≥3 (P Values)
Significant Efficacy is Underlined, Significant Worsening in *Italics*

	Day											
Group Comparison	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**
Aqueous Buffer Control v. DMSO Control	1.000	1.000	1.000	*0.024*	0.114	0.196	<0.001	0.002	<0.001	0.005	0.121	0.420
DMSO Control v. 250 mg/ml DHA in DMSO	1.000	1.000	*0.018*	*<0.001*	0.226	0.043	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*0.013*	*0.002*
Aqueous Buffer Control v. 25 mg/ml DHA in Aqueous Buffer	1.000	1.000	1.000	1.000	0.044	0.355	0.113	0.184	0.008	0.069	0.004	0.429
Aqueous Buffer Control v. 250 mg/ml DHA in Aqueous Buffer	1.000	1.000	1.000	1.000	0.422	1.000	1.000	0.170	0.652	1.000	0.448	0.170
Aqueous Buffer Control v. 250 mg/ml Ascorbic Acid in Aqueous Buffer	1.000	1.000	1.000	1.000	0.218	0.330	0.017	0.081	0.016	0.122	0.038	1.000

The 250 mg/ml DHA in DMSO Group.

Like the DMSO control group, this group showed early mucositis; by day 12 the severity of the mucositis was greater than the control. The mucositis peaked on day 14, but remained elevated for much of the remainder of the study. Examination of the extent of ulceration in this group showed a large and significant worsening of mucositis induced by this treatment. From day 12 to day 22, all animals in the group had ulcerations. Over the course of the entire study, animals in this group spent 70.3% of the time with ulcerations, a significant increase over the DMSO control ($p<0.001$; Table 3). The worsening of mucositis occurred throughout the study (days 12-28) as determined both by rank sum analysis (Table 1) and by chi-square analysis of duration of ulceration (Table 2).

Table 3

Animal Days with Scores ≥3
All Scores from Two Observers Counted
Scores Indicating Significant Efficacy are Underlined
Scores Indicating Significant Worsening are in *Italics*

Group	Days ≥3	Days <3	Total Days	% Days ≥3	Chi Sq v Control	P Value
250 mg/ml DHA in DMSO	135	57	192	70.3	80.932**	*<0.001***
Vehicle Control DMSO	46	146	192	24	16.748*	<0.001*
25 mg/ml DHA in Aqueous Buffer	45	147	192	23.4	17.658	<0.001*
250 mg/ml DHA in Aqueous Buffer	68	100	168	40.5	0.703	0.402*
Vehicle Control Aqueous Buffer	66	78	144	45.8	—	—
250 mg/ml Ascorbic Acid in Aqueous Buffer	41	127	168	24.4	14.864	<0.001*

Note: * indicates a chi square comparison with the aqueous buffer control group.
** indicates a chi square comparison with the DMSO control group.

The Aqueous Treatment Groups.

The mean daily mucositis scores were determined for the groups treated with aqueous solutions of 25 mg/ml DHA, 250 mg/ml DHA and 250 mg/ml ascorbic acid. In all three groups there was no worsening of mucositis as was observed in the 250 mg/ml DHA in DMSO group. Of the three groups, treatment with 25 mg/ml DHA group appeared to reduce the severity of mucositis. The 250 mg/ml ascorbic acid groups also shows a reduction of mucositis, while the 250 mg/ml DHA group shows a progression of mucositis that is very close to that observed in the aqueous buffer control group.

These observations are supported by analysis of the degree of severity of the mucosal lesions as indicated by a score of 3 or more. In this analysis, both the 25 mg/ml DHA and the 250 mg/ml ascorbic acid show an overall reduction in the frequency of ulcerations predominately on the healing side of the mucositis curve. The 25 mg/ml DHA group spent 23.4% of the study with lesions, a significant reduction ($p<0.001$) when compared to the 45.8% of days with lesions in the control group (Table 3). Animals treated with 250 mg/ml ascorbic acid group spent 24.4% of the study time with ulcerations, also a significant reduction when compared to the control group ($p<0.001$). The period of improvement for both 25 mg/ml DHA and 250 mg/ml ascorbic acid groups was during the late healing phase (days 22-26) as determined both by rank sum analysis of all scores (Table 1) or by chi-square analysis of the distribution of scores greater than or equal to 3 (Tables 2 and 3).

At the higher dose of 250 mg/ml DHA, there was no significant change in the course of mucositis, this group was indistinguishable form the control group by every statistical measurement made in this study.

Conclusions

Survival was unaffected by topical administration of test compounds.

DMSO adversely affected weight gain when administered topically. It seems likely that this finding was the consequence of the effect of the compound on appetite, rather than a reflection of true toxicity as there was no wasting of animals or signs of gastrointestinal injury. This observation was unaffected by the inclusion of DHA.

Topical administration of DMSO resulted in a reduction in the overall severity and duration of radiation-induced mucositis compared to sham therapy with aqueous buffer. Although a favorable effect was noted during the developmental phases of mucositis, no difference in the peak mucositis score was noted between DMSO and aqueous buffer treated

animals. However, mucositis induction was slightly delayed in the DMSO group and resolved more quickly. While it appears that DMSO is of benefit at the level of injury, its smell, effect on weight and noxious potential likely preclude its possible usefulness as a vehicle for topical oral administration.

The addition of 250 mg/ml DHA to DMSO profoundly worsened the course and severity of mucositis. The dose of DHA in this vehicle was clearly not optimal. Based on the dose response observed for DHA in aqueous buffer, it is quite possible that DHA in a lower concentration (25 mg/ml) in DMSO would impart additional efficacy in an animal model.

DHA at concentrations of 25 mg/ml in aqueous buffer demonstrated significant efficacy on the course and duration of mucositis, significantly reducing the overall duration of clinically significant lesions by close to 50%. The effect of DHA was most notable during the very early stages of mucositis development and during the healing phase of the condition. Interestingly, a reverse dose-response was seen. An increase in DHA concentration to 250 mg/ml in an aqueous formulation produced results that were no better than the control.

The administration of ascorbic acid (250 mg/ml) in aqueous buffer yielded results relative to mucositis that were comparable to those seen with 25 mg/ml of DHA and was significantly better than the control.

The results obtained in this study suggest that DHA and ascorbic acid in aqueous buffer may be of benefit as an intervention for mucositis. While DMSO may be of value also, its current physical characteristics may preclude its clinical utility.

Example 5: Treatment of stroke/reperfusion injury

To determine the effect of buffered dehydroascorbic acid compositions on the injuries resulting from ischemia, buffered DHA formulations were administered to mice in a well-studied rodent model of ischemic stroke. Nonreperfused ischemia was created by intraluminal middle cerebral artery occlusion in mice and standard indicia of ischemia were measured as previously described (Connolly et al., *Neurosurgery*. 38(3)523-532, 1996).

Buffered dehydroascorbic acid compositions for intravenous use were prepared as described above in Example 3A. Infarct volumes were determined by staining cerebral sections with triphenyl tetrazolium chloride (TTC) and performing digital image analysis as previously described (Huang et al., *Science* 285:595-599, 1999). The same TTC stained sections were also analyzed by an indirect method (Lin et al., *Stroke* 24:117-121, 1993). Neurologic deficit was determined using a four-tiered grading system, intracerebral

hemorrhage was determined spectrophotometrically, and cerebral blood flow was measured by laser Doppler flowmetry as previously described (Huang et al., 1999). Cerebral blood flow, intracerebral hemorrhage, neurological score, infarct volume (% ipsilateral hemisphere) and mortality were compared using Student's t-test.

Dehydroascorbic acid formulations of 250 mg/kg dehydroascorbic acid (DHA-250) or 500 mg/kg dehydroascorbic acid (DHA-500) were administered 15 minutes or 3 hours after permanent ischemia. Both postischemic DHA regimens protected animals similarly: neurological deficit was decreased, infarct volume was reduce 6- to 9-fold without any increase in intracerebral hemorrhage, and mortality was reduced 66% ($p<0.05$). As shown in Fig. 1, DHA-250 ($p=0.04$) and DHA-500 ($p=0.003$) administered 3 hours postischemia significantly reduced infarct volume relative to 500 mg/ml ascorbic acid (AA-500). DHA-500 also significantly reduced infarct volume relative to vehicle control ($p=0.01$). As shown in Fig. 2, similar results were obtained when the infarct volumes were measured using a digital or "direct" method. Fig. 3 shows that administration of DHA-250 or DHA-500 3 hours postischemia increased relative cerebral blood flow. Fig. 4 demonstrates that neurological scores were significantly increased following administration of DHA-250 or DHA-500 3 hours postischemia. DHA-250 significantly improved neurological score as compared to AA-500 ($p=0.024$). DHA-500 significantly improved neurological score as compared to AA-500 or vehicle control ($p=0.004$ and $p=0.031$, respectively). Mortality also was reduced in a dose-dependent manner when DHA was administered 3 hours postischemia (Fig. 5).

These results demonstrate that postischemic administration of DHA is effective in reducing the effects of ischemia, and furthermore suggest that treatment thresholds longer than 3 hours are feasible with DHA. In contrast, administration of ascorbic acid did not improve neurological function, infarct volume or survival in any of these experiments. It can be concluded that DHA confers dose dependent neuroprotection in ischemic stroke, even with delayed administration, and thus may be useful in the treatment of stroke in humans.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

All references disclosed herein are incorporated by reference.

What is claimed is:

Claims

1. A pharmaceutical composition comprising dehydroascorbic acid and a pharmaceutically acceptable buffering system, wherein the composition has a pH greater than about 3, and wherein the concentration of dehydroascorbic acid is at least about 5 mg/mL.

2. The composition of claim 1, wherein the pH is between about 3 and about 7.

3. The composition of claim 2, wherein the pH is between about 4 and about 6.

4. The composition of claim 3, wherein the pH is between about 4.5 and about 5.5.

5. The composition of claim 4, wherein the pH is between about 4.8 and about 5.2.

6. The composition of claim 5, wherein the pH is about 5.

7. The composition of claim 1, wherein the pharmaceutically acceptable buffering system comprises an alkaline or buffering agent selected from the group consisting of acetate, phosphate, citrate, glycine, borate, carbonate, bicarbonate, hydroxide and pharmaceutically acceptable salts thereof.

8. The composition of claim 7, wherein the alkaline or buffering agent is sodium bicarbonate or sodium acetate.

9. The composition of claim 1, further comprising a preservative.

10. The composition of claim 9, wherein the preservative is EDTA or sodium benzoate.

11. The composition of claim 1, wherein the concentration of dehydroascorbic acid is between about 10 mg/mL and about 1000 mg/mL.

12. The composition of claim 11, wherein the concentration of dehydroascorbic acid is between about 50 mg/mL and about 750 mg/mL.

13. The composition of claim 12, wherein the concentration of dehydroascorbic acid is between about 100 mg/mL and about 500 mg/mL.

14. The composition of claim 13, wherein the concentration of dehydroascorbic acid is between about 200 mg/mL and about 300 mg/mL.

15. The composition of claim 14, wherein the concentration of dehydroascorbic acid is about 250 mg/mL.

16. A method for treating a subject to increase the concentration of ascorbic acid in a tissue of the subject, comprising

administering to a subject in need of such treatment an amount of a buffered dehydroascorbic acid composition having a pH greater than about 3 effective to increase the concentration of ascorbic acid in the tissue.

17. The method of claim 16, wherein the pH is between about 3 and about 7.

18. The method of claim 17, wherein the pH is between about 4 and about 6.

19. The method of claim 18, wherein the pH is between about 4.5 and about 5.5.

20. The method of claim 19, wherein the pH is between about 4.8 and about 5.2.

21. The method of claim 20, wherein the pH is about 5.

22. The method of claim 16, wherein the concentration of dehydroascorbic acid is between about 5 mg/mL and about 1000 mg/mL.

23. The method of claim 22, wherein the concentration of dehydroascorbic acid is between about 50 mg/mL and about 750 mg/mL.

24. The method of claim 23, wherein the concentration of dehydroascorbic acid is between about 100 mg/mL and about 500 mg/mL.

25. The method of claim 24, wherein the concentration of dehydroascorbic acid is between about 200 mg/mL and about 300 mg/mL.

26. The method of claim 25, wherein the concentration of dehydroascorbic acid is about 250 mg/mL.

27. The method of claim 16, wherein the buffered dehydroascorbic acid composition is administered by a mode selected from the group consisting of topical, intravenous, oral, intracavity, intrathecal, intrasynovial, buccal, sublingual, intranasal, transdermal, intravitreal, subcutaneous, intramuscular and intrarectal lavage.

28. The method of claim 27, wherein the buffered dehydroascorbic acid composition is administered as a bolus intravenous injection.

29. The method of claim 27, wherein the buffered dehydroascorbic acid composition is administered as an intravenous infusion.

30. The method of claim 16, wherein the subject has a condition associated with oxidative stress.

31. The method of claim 30, wherein the oxidative stress results in damage to an epithelial tissue of the subject, wherein the buffered dehydroascorbic acid composition is administered to an apical surface of the epithelial tissue.

32. The method of claim 30, wherein the condition associated with oxidative stress is selected from the group consisting of congestive heart failure, atherosclerosis, neurodegenerative disorders, familial adenomatous polyposis, celiac disease, alcoholic liver disease, inflammatory disease, diabetes, cystic fibrosis, ischemic reperfusion injury, subarachnoid hemorrhage, prion disease, multiple sclerosis, and hyperthyroidism.

33. The method of claim 32, wherein the neurodegenerative disorder is selected from the group consisting of Parkinson's disease, Alzheimer's disease, Huntington's disease,

amyotrophic lateral sclerosis, presenile dementia, spongiform encephalopathy, and behavioral disorders.

34. The method of claim 32, wherein the inflammatory disease is selected from the group consisting of inflammatory bowel disease , rheumatoid arthritis and pancreatitis.

35. The method of claim 34, wherein the inflammatory bowel disease is selected from the group consisting of Crohn's disease and colitis.

36. The method of claim 32, wherein the prion disease is selected from the group consisting of Creutzfeld-Jakob disease, new variant Creutzfeld-Jakob disease, bovine spongiform encephalopathy and scrapie.

37. The method of claim 32, wherein the ischemic reperfusion injury is stroke.

38. The method of claim 16, wherein the subject has a coronary heart disease.

39. A method for *ex vivo* preservation of a tissue or organ, comprising

contacting the tissue or organ *ex vivo* with an amount of a buffered dehydroascorbic acid composition having a pH greater than about 3 effective to increase the concentration of ascorbic acid in the tissue or organ.

40. The method of claim 39, wherein the pH is between about 3 and about 7.

41. The method of claim 40, wherein the pH is between about 4 and about 6.

42. The method of claim 41, wherein the pH is between about 4.5 and about 5.5.

43. The method of claim 42, wherein the pH is between about 4.8 and about 5.2.

44. The method of claim 43, wherein the pH is about 5.

45. The method of claim 39, wherein the concentration of dehydroascorbic acid is between about 5 mg/mL and about 1000 mg/mL.

46. The method of claim 45, wherein the concentration of dehydroascorbic acid is between about 50 mg/mL and about 750 mg/mL.

47. The method of claim 46, wherein the concentration of dehydroascorbic acid is between about 100 mg/mL and about 500 mg/mL.

48. The method of claim 47, wherein the concentration of dehydroascorbic acid is between about 200 mg/mL and about 300 mg/mL.

49. The method of claim 48, wherein the concentration of dehydroascorbic acid is about 250 mg/mL.

50. The method of claim 39, wherein the composition further comprises a standard organ perfusion fluid selected from the group consisting of University of Wisconsin solution, Euro-Collins solution, BT01 solution, Ringer's lactate solution and normal saline solution.

51. The method of claim 39, wherein the composition further comprises an organ perfusion agent selected from the group consisting of calcium entry blockers including lidoflazine; cytoprotectors including natriuretic factor, PGI2 and trimetazidine; free radical chelating agents and scavengers including allopurinol, mannitol and glutathione; and substrates for the mitochondrial respiratory chain including aspartate and glutamate.

52. A method for treating a condition involving unwanted free radicals comprising administering to a cell or tissue of a subject afflicted by such a condition an amount of a buffered dehydroascorbic acid composition having a pH greater than about 3 effective to reduce free radicals in the cell or tissue.

53. The method of claim 52, wherein the condition is selected from the group consisting of cancer, cardiovascular disease and cataracts.

54. The method of claim 52, wherein the pH is between about 3 and about 7.

55. The method of claim 54, wherein the pH is between about 4 and about 6.

56. The method of claim 55, wherein the pH is between about 4.5 and about 5.5.

57. The method of claim 56, wherein the pH is between about 4.8 and about 5.2.

58. The method of claim 57, wherein the pH is about 5.

59. The method of claim 52, wherein the concentration of dehydroascorbic acid is between about 5 mg/mL and about 1000 mg/mL.

60. The method of claim 59, wherein the concentration of dehydroascorbic acid is between about 50 mg/mL and about 750 mg/mL.

61. The method of claim 60, wherein the concentration of dehydroascorbic acid is between about 100 mg/mL and about 500 mg/mL.

62. The method of claim 61, wherein the concentration of dehydroascorbic acid is between about 200 mg/mL and about 300 mg/mL.

63. The method of claim 62, wherein the concentration of dehydroascorbic acid is about 250 mg/mL.

64. A pharmaceutical composition comprising

dehydroascorbic acid, and

a non-dehydroascorbic acid therapeutic agent, together in an amount effective for treating a condition.

65. The composition of claim 64, further comprising a pharmaceutically acceptable buffering system, wherein the composition has a pH greater than about 3.

66. The composition of claim 65, wherein the concentration of dehydroascorbic acid is at least about 5 mg/mL.

67. The composition of any of claims 64-66, wherein the non-dehydroascorbic agent is selected from the group consisting of antibacterial agents, antifungal agents, antiviral agents, analgesics, non-DHA anti-mucositis agents, antistroke agents, anticancer agents and antineurodegenerative agents.

68. The composition of claim 67, wherein the antistroke agent is selected from the group consisting of antiplatelet agents, anticoagulation agents, thrombolytic agents including plasminogen activators, antithrombotics, neuroprotective agents, platelet activating factor antagonists, platelet aggregation inhibitors, post-stroke and post-head trauma treatments, cerebral ischemia agents, basic fibroblast growth factors and steroids.

69. A medical product comprising
an isolated organ in a perfusion fluid containing dehydroascorbic acid.

70. A medical product comprising an organ perfusion fluid containing dehydroascorbic acid.

71. A method for reducing the *in vivo* toxicity of a dehydroascorbic acid pharmaceutical composition, comprising
buffering the dehydroascorbic acid composition to a pH of at least about 3.

72. A method for treating mucositis in a tissue of a subject, comprising
administering to a subject in need of such treatment an amount of a dehydroascorbic acid composition effective to reduce mucositis in the tissue.

73. The method of claim 72, wherein the concentration of dehydroascorbic acid is between about 5 mg/mL and about 1000 mg/mL.

74. The method of claim 73, wherein the concentration of dehydroascorbic acid is between about 50 mg/mL and about 750 mg/mL.

75. The method of claim 74, wherein the concentration of dehydroascorbic acid is between about 100 mg/mL and about 500 mg/mL.

76. The method of claim 75, wherein the concentration of dehydroascorbic acid is between about 200 mg/mL and about 300 mg/mL.

77. The method of claim 76, wherein the concentration of dehydroascorbic acid is about 250 mg/mL.

78. The method of claim 72, wherein the dehydroascorbic acid composition is a buffered dehydroascorbic acid composition having a pH greater than about 3.

79. The method of claim 78, wherein the pH is between about 3 and about 7.

80. The method of claim 79, wherein the pH is between about 4 and about 6.

81. The method of claim 80, wherein the pH is between about 4.5 and about 5.5.

82. The method of claim 81, wherein the pH is between about 4.8 and about 5.2.

83. The method of claim 82, wherein the pH is about 5.

84. The method of claim 72, wherein the mucositis is caused by radiation therapy or chemotherapy.

85. The method of claim 72, wherein the mucositis is oral mucositis.

86. The method of claim 72, wherein the composition is administered topically.

87. The method of claim 72, further comprising administering to the subject at least one compound selected from the group consisting of antibacterial agents, antifungal agents, antiviral agents, analgesics and non-DHA anti-mucositis agents.

88. A method for reducing the side effects of an anticancer therapeutic treatment comprising

administering to a subject in need of such treatment an effective amount of a dehydroascorbic acid composition to reduce the side effects, wherein the dehydroascorbic acid composition is administered in combination with the anticancer therapeutic treatment.

89. The method of claim 88, wherein the dehydroascorbic acid composition is a buffered dehydroascorbic acid composition having a pH greater than about 3.

90. The method of claim 88, wherein the dehydroascorbic acid composition is administered substantially simultaneously with the anticancer therapeutic treatment.

91. The method of claim 88, wherein the dehydroascorbic acid composition is administered prophylactically prior to the administration of the anticancer therapeutic treatment.

92. The method of claim 88, wherein the anticancer therapeutic treatment is a chemotherapeutic agent.

93. A method for treating tissue injury associated with ulcers of the mouth, pharynx or gastrointestinal tract of a subject, comprising

administering to a subject in need of such treatment an amount of a dehydroascorbic acid composition effective to reduce tissue injury associated with ulcers of the mouth, pharynx or gastrointestinal tract.

94. The method of claim 93, wherein the dehydroascorbic acid composition is a buffered dehydroascorbic acid composition having a pH greater than about 3.

95. The method of claim 93, wherein the concentration of dehydroascorbic acid is between about 5 mg/mL and about 1000 mg/mL.

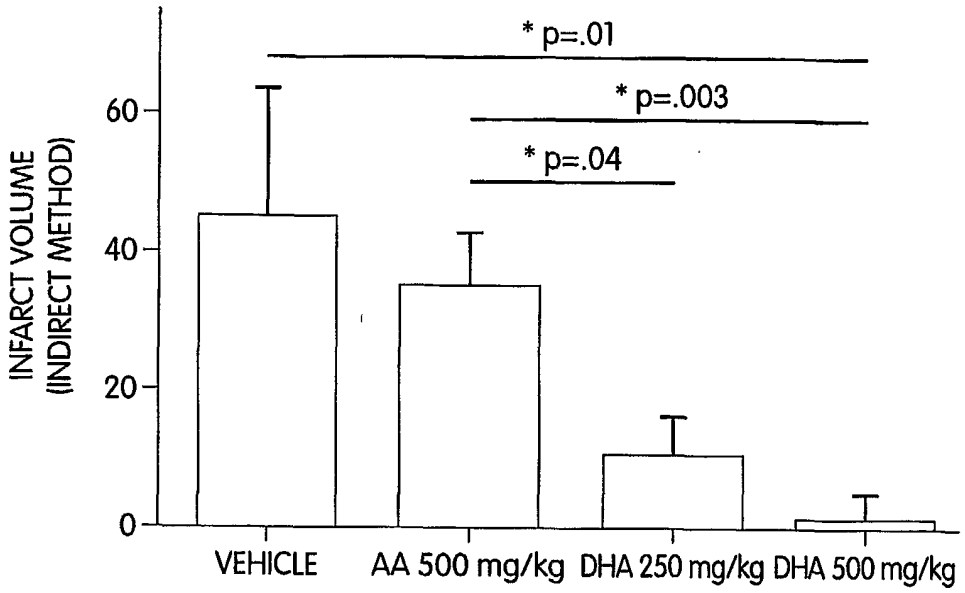

* p=.01
* p=.003
* p=.04
INFARCT VOLUME
(INDIRECT METHOD)
60
40
20
0
VEHICLE
AA 500 mg/kg
DHA 250 mg/kg
DHA 500 mg/kg

Fig. 1

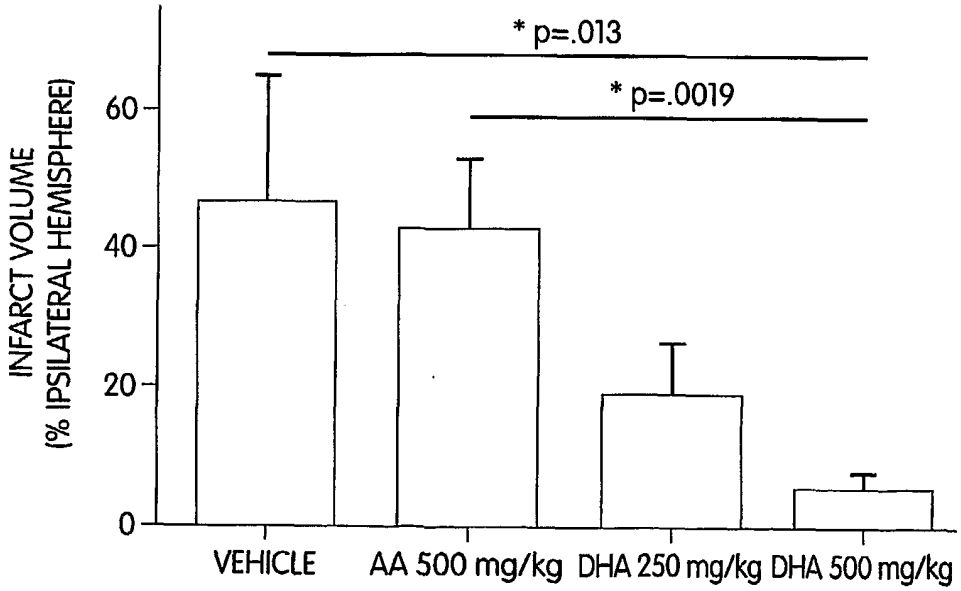

* p=.013
* p=.0019
INFARCT VOLUME
(% IPSILATERAL HEMISPHERE)
60
40
20
0
VEHICLE
AA 500 mg/kg
DHA 250 mg/kg
DHA 500 mg/kg

Fig. 2

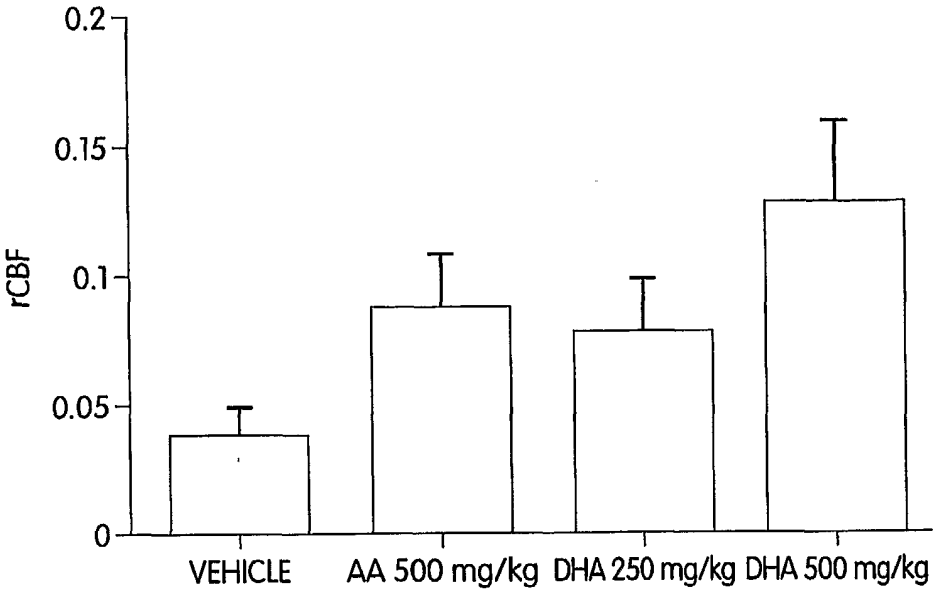

0.2
0.15
0.1
0.05
0
rCBF
VEHICLE
AA 500 mg/kg
DHA 250 mg/kg
DHA 500 mg/kg

Fig. 3

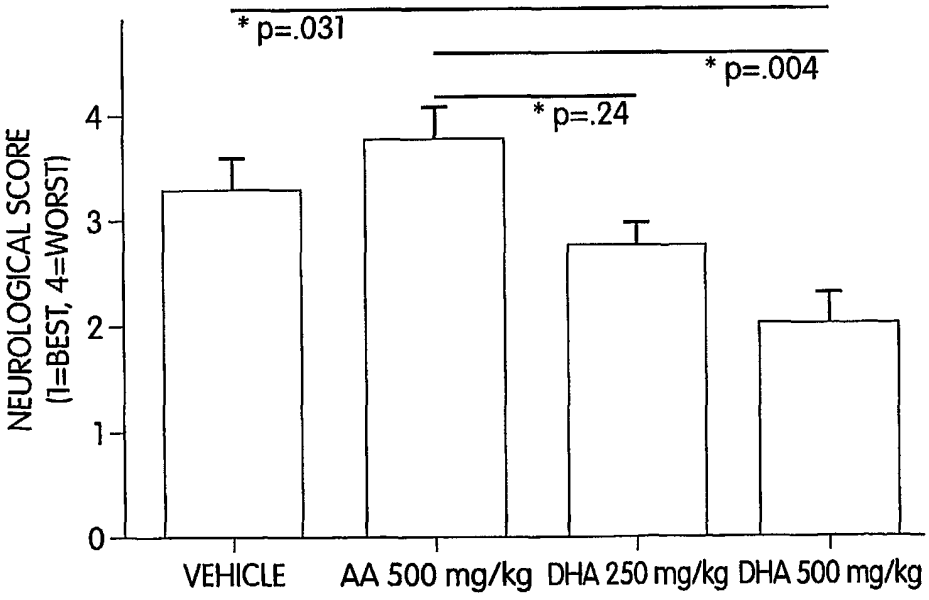

* p=.031
* p=.004
* p=.24
4
3
2
1
0
NEUROLOGICAL SCORE
(1=BEST, 4=WORST)
VEHICLE
AA 500 mg/kg
DHA 250 mg/kg
DHA 500 mg/kg

Fig. 4

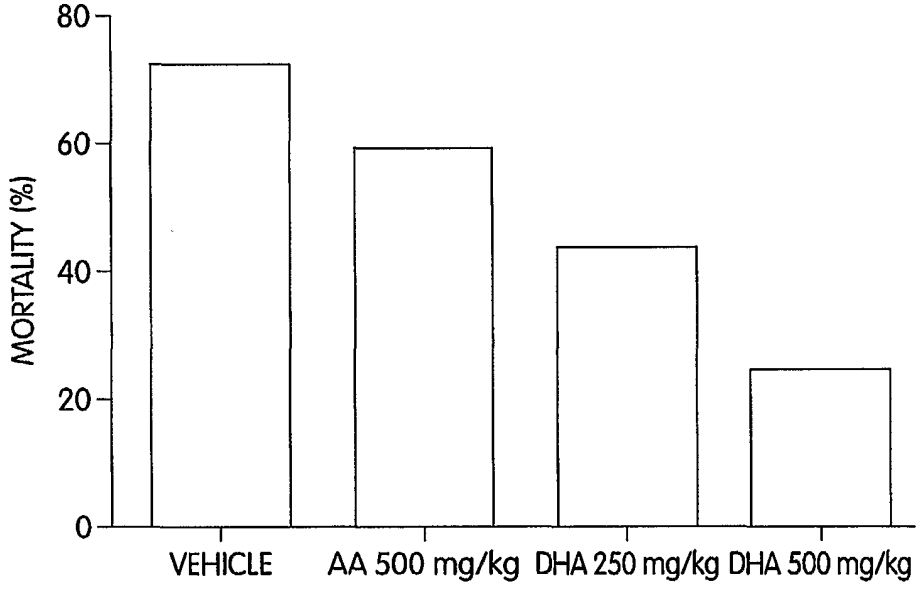

80
60
40
20
0
MORTALITY (%)
VEHICLE
AA 500 mg/kg
DHA 250 mg/kg
DHA 500 mg/kg

Fig. 5