Cancer Therapy: A Review with Scientific Validation for the Role of Electronically Modified Oxygen Derivatives in Oncologic Treatment Modalities

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Abstract

The American Cancer Society and the British Columbia Cancer Agency state that electronically modified oxygen derivatives, such as hydrogen peroxide and other "oxidative therapies," are basically ineffective, harmful or even lethal in the treatment of cancer. A compelling body of evidence over the past few decades demands that the therapeutic role of oxygen derivatives be reevaluated. The free radical theory defined oxygen free radicals or reactive oxygen species as being destructive and as the cause of the majority of common human diseases. Yet, decades of experimentation have shown that the free radical theory lacks predictability, fails to meet the requirements of the scientific method and is therefore invalidated. This nullification requires reexamination of oxidative oncologic complementary, alternative and integrative treatment modalities. Prooxidants, some of which are oxygen free radicals or reactive oxygen species, have been blamed for cancer causation and unscrupulous marketers have brought discredit to oxygen based therapies and disregard to oxidative centered treatments. In contrast, a review of currently effective tumoricidal methods reveals a "commonality of oxygen based, anti-neoplastic action," in that many successful cytotoxic agents, procedures or methods have been shown to proceed primarily via prooxidants. Discussions will compare chemotherapy, radiation therapy, megadose intravenous vitamin C therapy, photodynamic therapy, sonodynamic therapy, the Howes' singlet oxygen tumoricidal system, ozone therapy, hyperbaric oxygen therapy and hydrogen peroxide therapy. Various prooxidant delivery systems currently offer beneficial, unique tumoricidal properties and approach the "Holy Grail" for cancer treatments, allowing for selective killing of cancer cells while sparing normal cells. This review describes these prooxidant EMOD agents and areas of possible complementarity of oxidative therapies (prooxidant stacking) based on the available scientific literature. Decades of scientific study have shown that prooxidant antineoplastic therapeutic agents provide significant clinical advantage and offer safe, effective and economical promise in the future treatment of cancer.

INTRODUCTION

The widely held flawed notions promulgated by the free radical theory have so biased world orthodoxy, regarding the true role of oxygen in disease causation and prevention that it is best to start over with a new, well configured, open minded scientific paradigm. To this end, prior "oxidative" prejudicial terminology will be eschewed.

Unproven therapies and misrepresented products, offered over the internet and at various clinics, local and abroad, have created a generalized negative attitude towards socalled "oxygen therapies" and "oxidative medicine," because it has been used to refer to any number of worthless products or ineffective treatments, which were not based on scientific facts regarding oxygen metabolism. The therapeutic potential of prooxidant electronically modified oxygen derivatives (EMODs) have been demonstrated for decades by their use in academic oncology treatment programs. Many so-called oxidative therapies prompted the British Columbia Cancer Agency (a part of the Canadian Provincial Health Services Authority) and the American Cancer Society (ACS) to recommend against their use. These therapies go by many names including "Oxygen Therapies, Hyperoxygenation Therapy, Oxymedicine, Bio-Oxidative Therapy, Oxidative Therapy, Oxidology, Ozone therapy, Autohemotherapy, Hydrogen peroxide therapy and Germanium sesquioxide therapy." To be sure, some of these approaches are subject to fraudulent practices and lack credibility but others are based on a solid scientific principles and investigations. (ACS website accessed 12-7-09).

The BC Cancer Agency website presents the following summary: "Patients with cancer should not consider oxygen

therapies as either alternative (first-line) or adjunct (complementary) therapies. Researchers now understand that cancer cells "lower-than-normal respiration" is due to the fact that tissue surrounding cancer cells receives less oxygen because it has fewer blood vessels feeding it. Oxygen therapies have not been found useful against cancer and are not used as mainstream cancer treatments." They also state that, "Oxygen therapy can destroy cells, including those of the blood-forming organs. Very high doses can seriously damage health or even cause death." (BC Cancer Agency website accessed 8-31-09).

The American Cancer Society website gives the following overview: "Available scientific evidence does not support claims that putting oxygen-releasing chemicals into a person's body is effective in treating cancer. It may even be dangerous. There have been reports of patient deaths from this method."

Contrary to disputed statements of major cancer agencies, this review clearly demonstrates that prooxidant EMODs have been scientifically confirmed as essential, effective and safe clinical agents in the battle against cancer. Undeniably, for decades, prooxidant EMOD cancer therapeutic modalities have been a mainstay for our most effective oncologic treatment programs, which utilize chemotherapy, radiation therapy and photodynamic therapy. Ignorance of the literature does not allow health care agencies or others the latitude of making scientifically unsupported statements.

Simultaneously, a persuasive assemblage of scientific data shows that EMODs are crucial agents for gene regulation, maintenance of cellular oxidation/reduction (redox) homeostasis and pathogen and neoplasia protection.

At first glance, oxygen has obvious medical benefits in emergency or critical care situations but upon closer review of the available scientific literature, it becomes readily apparent that EMODs have already made significant contributions in fighting disease, maintaining healthy homeostasis and in combatting cancer. As it relates to cancer therapy, prooxidant EMOD-induced apoptosis and necrosis is currently used in a wide spectrum of modalities to successfully treat neoplasia. There appears to be "a prooxidant point of convergence" in these EMOD applications, which includes a role in chemotherapy, radiation therapy, intravenous vitamin C mega-dose therapy, photodynamic therapy, sonodynamic therapy, the Howes singlet oxygen tumoricidal system, ozone therapy, hyperbaric oxygen therapy and intravenous hydrogen peroxide therapy.

Conversely, hypoxia and so-called antioxidants can effectively modify or block cancer cell kill by interfering with electronically modified oxygen derivative (EMOD)induced apoptosis. EMODs possess the levels of reactivity to serve as tumoricidal agents.

Direct reactions of dioxygen are slow for two reasons: 1) ground state dioxygen is a triplet state and most reactants are singlets and 2) triplet-to-singlet spin conversions are forbidden by quantum mechanics and are thus slow.

Ground state triplet dioxygen (O_2) does not have the same level of reactivity as the prooxidants referred to in this article, such as: the superoxide anion (O_2 .-), hydrogen peroxide (H_2O_2), metastable excited singlet oxygen (1O_2*), the hydroxyl radical (OH.), hypochlorous acid (HOCl), nitric oxide (NO), peroxynitrite (OONO-), ozone (O_3), etc. However, ground state triplet oxygen serves as the source (the precursor) for the production of the entire family of EMOD agents. EMODs are formed by basic alterations of the electron structure of ground state triplet oxygen, such as addition or removal of electrons, altered electron spin configurations, altered pi electron orbital positions, combinations with nitrogen, exposure to ultraviolet light or wave specific white light, altered pressure other than atmospheric, etc.

Gathering EMOD agents into inaccurate and misleading categorizations is no longer suitable with the use of terms such as oxygen free radials or reactive oxygen species. The usage of incorrect biochemical terminology is no longer acceptable and its taint must be abandoned. As Carl Nathan said in a 2003 Journal of Clinical Investigation article, "terms of discourse" need to be addressed.

Because of the common use of varying terms, such as reactive oxygen species (ROS), reactive oxygen intermediates (ROI), reactive oxygen metabolites (ROM), active oxygen species (AOS), oxygen species (OS), etc., confusion abounds as to precise nature of the oxygen entities being discussed in various articles. Thus, in 2005, in The Medical and Scientific Significance of Oxygen Free Radical Metabolism. pg. 39, I stated, "It is also time to discard ROS, RONS, OS, ROI, ROM, AOS, etc. and utilize a more meaningful and accurate term. I proposed the term "electronically modified oxygen derivative(s)" (EMODs). This term does not imply charge, radicality, or reactivity. It merely indicates that an electron(s) of oxygen has (have) been altered or changed from its ground state orbit. This avoids all of the inaccuracies of terms such as reactive oxygen species, reactive oxygen metabolites, or oxygen intermediates, all of which should be discarded from usage. Thus, EMODs include superoxide anion, singlet oxygen, hydrogen peroxide, hypochlorous acid, peroxynitrite, hydroxyl radical, nitric oxide, alkyl radicals, alkoxyl radicals, etc. The term does not limit itself to oxygen covalent bonding or hydrogen abstraction and addition. Thus, oxygen-containing sulfates, nitrates, phosphates, etc. would also qualify as EMODs. Further, it includes all of the nitrative and oxidative forms of oxygen."

Further, according to Barry Halliwell, EMODs such as superoxide anion are barely "reactive" at all and are redox ambivalent at a physiological pH. Additionally, EMODs, such as hydrogen peroxide, singlet oxygen, ozone and hypochlorous acid are not free radicals but are frequently erroneously placed in this chemical category.

In 1971, President Nixon launched the "War Against Cancer," which was designed to fight the escalating incidence of cancer that had assumed epidemic proportions. According to Samuel S. Epstein's book, Cancer-GATE: How to Win the Losing Cancer War, only incremental progress has been made in this overall crusade. The development of agents that improve or enhance the efficacy of cancer therapy is one of the most important areas of research in current medical oncology. Biological oxidation/reduction (redox) reactions are central to metabolism, cellular energy production and cancer therapy.

Many in vitro studies have shown support of prooxidant cancer therapies and even though it should not be assumed that they will be identically effective in vivo in the cure of cancer, clinical studies cited in this review are increasingly showing support for this thesis.

DISCUSSION HARMAN'S FREE RADICAL THEORY

Harman's free radical theory hypothesized that diseases, such as cancer and aging, resulted from the random or "stochastic" accumulation of oxidative damage purportedly caused by EMODs, from environmental sources and from by-products of normal cellular metabolism.¹⁻⁵ When investigators found that their results were not as predicted by the free radical theory, they either discounted their results or referred to them as a paradox. Countless examples of this are in the literature but can be best illustrated by the 1995 tome edited by Kelvin J.A. Davies and Fulvio Ursini entitled, THE OXYGEN PARADOX.

The alleged damaging derivatives of oxygen were defined as being inherently deleterious and harmful. However, this notion has been rebuffed by Howes.⁶ Apoptosis, necrosis, and growth arrest have been shown to be regulated to a significant degree by prooxidant EMOD species.⁷⁻¹⁰ Apoptosis, in part, controls the neoplastic process as genetically damaged or mutated cells can be eliminated by inducement of the apoptotic process.¹¹ Apoptosis involves caspases (cysteine proteases cleaving after particular aspartate residues), mitochondrial pathways and/or EMODs, which are usually, but not always, key components.¹² Many apoptosis-inducing agents function as prooxidants in vitro.¹³

Prooxidant EMOD generating agents have repeatedly been shown to kill cancer cells selectively, while sparing normal cells and this tumoricidal action can be modified or blocked by antioxidants, which may accelerate cancer growth both in vitro and in vivo.¹⁴⁻¹⁷ Since therapeutic agents (radiation therapy, chemotherapy or photodynamic therapy, PDT) work, to a considerable extent, by releasing prooxidant free radicals (EMODs), it is logical that antioxidants likely interfere with their action. EMOD levels and cellular redox tone appear to be uniquely exploitable targets in cancer chemoprevention via the stimulation or induction of cytoprotection in normal cells and/or the induction of apoptosis in transformed malignant cells.

ANTIOXIDANTS AND APOPTOSIS

Yet, some believe that antioxidants may play a central role in apoptosis and cancer therapy. Some investigators have made claims that antioxidants can actually kill cancer cells and argue that antioxidants are beneficial during chemotherapy. A review on the use of antioxidants during chemotherapy, published in Cancer Treatment Reviews, was a collaborative effort led by Dr. Keith Block and researchers from the University of Illinois at Chicago and M.D. Anderson Cancer Center in Houston. After reviewing articles, only 33 of 965 articles considered, including 2,446 subjects, met the inclusion criteria. Antioxidants evaluated were: glutathione, melatonin, vitamin A, an antioxidant mixture, N-acetylcysteine, vitamin E, selenium, L-carnitine, Co-Q10 and ellagic acid. Nine studies reported no difference in toxicities between the 2 groups. Only 1 study (vitamin A) reported a significant increase in toxicity in the antioxidant

group. This review provides some evidence that antioxidant supplementation during chemotherapy might reduce doselimiting toxicities but it must be kept in mind that many of these so-called antioxidants have considerable prooxidant activity to which their salutary effects could also be attributed. Larger, well-designed studies of antioxidants impact on PDT, chemotherapy and tumoricidal radiation therapy are warranted.¹⁸

However, until such data is available, considerations for utmost patient safety must prevail. The mechanisms of action of chemotherapeutic drugs and antioxidants are sufficiently understood to predict their resultant interactions and to suggest that considerable care should be exercised with respect to both clinical decisions and study interpretations. ¹⁹ Additionally, antioxidants have a wide variety of biochemical actions and are capable of interfering selectively with EMOD initiation, propagation and termination. EMODs have been studied for their positive effects in the prevention or cure of many cancers, cardiovascular disease, age-related diseases, and other disorders. ²⁰⁻²³

Nonetheless, there seems to be agreement that the antioxidant N-acetylcysteine (NAC), a derivative of the naturally occurring amino acid cysteine, should be avoided by cancer patients because of studies showing interference with chemotherapeutic agents, such as cisplatin and doxorubicin.^{24, 25} A 2005 report concluded that cancer patients should avoid antioxidant supplements while receiving chemotherapy or radiation treatment.²⁶ Directed towards informing the public, a Wall Street Journal article argued that antioxidants could block the beneficial effects of standard cancer therapy.²⁷

Those who recommend the use of antioxidants in cancer patients claim that antioxidants such as vitamin C, vitamin E, coenzyme Q10, glutathione, and selenium can reduce the toxicity of free radicals.²⁸⁻³¹ Thus, EMOD-induced prooxidant apoptosis and the cancer conundrum leave us with unanswered questions regarding their interactions, auto-oxidation of antioxidants and the prooxidant character of many antioxidants.³²

A 2007 article not only defends the use of antioxidants in cancer patients, it states that, "In 15 human studies, 3,738 patients who took non-prescription antioxidants and other nutrients actually had increased survival." ³³ In contrast, a 2008 article in the Journal of the National Cancer Institute

reviewed randomized trial data, which suggested that cancer patients should avoid the routine use of antioxidant supplements because they may potentially decrease the efficacy of cancer therapy by protecting the tumor and reducing survival. They looked at clinical trials investigating the impact of antioxidants on radiation therapy and found evidence suggesting that antioxidant supplementation reduced overall survival.³⁴

HYPOXIA (LOW OXYGEN LEVELS) THRESHOLD LEVELS OF OXYGEN (O)

Hypoxia (defined as the fraction of measured O_2 partial pressures of <5 mmHg) is a statistically significant adverse prognostic factor of disease-free survival. Considerable data indicates that low O_2 in tumor cells is an adverse prognostic sign. In general, low tumor O_2 is associated with: increased aggressiveness of primary cancerous lesions, their ability to metastasize, and an increased resistance to treatments with irradiation, chemotherapeutics and surgery.

In general, median O_2 partial pressures of less than 10 mmHg result in intracellular acidosis, ATP depletion, a drop in the energy supply and increasing levels of inorganic phosphate. Mitochondrial oxidative phosphorylation is limited at O_2 partial pressures of less than approximately 0.5 mmHg but there are exceptions to this generality.

Overall, a number of key findings have been described as follows: 1) most tumors have lower median O_2 partial pressures than their tissue of origin; 2) many solid tumors contain areas of low O_2 partial pressure than cannot be predicted by clinical size, stage, grade, histology and site; 3) tumor-to-tumor variability in oxygenation is usually greater than intra-tumoral variability in oxygenation; and 4) recurring tumors have a poorer oxygenation status than the corresponding primary tumors.

Cancer cell apoptosis or cellular suicide (apoptotic execution) is considered to be a needed means for controlling the growth or proliferation of neoplastic cells, which is highly desirable and the goal of cancer therapy.

Tumor hypoxia and oxygen deficiency is strongly implicated in the growth of tumors and is a known adverse factor in the effectiveness of conventional radiation and chemotherapy.^{35.} ³⁶ Hypoxia can induce programmed (apoptotic) cell death in normal and neoplastic cells. The level of p53 in cells increases under hypoxic conditions, and the increased level of p53 induces apoptosis by a pathway involving Apaf-1 and caspase-9 as downstream effectors.³⁷ However, hypoxia also initiates p53-dependent apoptosis pathways involving hypoxia-inducible factor-1 (HIF-1), genes of the BCL-2 family, and other unidentified genes.³⁸

Hypoxia stimulates the transcription of glycolytic enzymes, glucose transporters (GLUT1 and GLUT3), angiogenic molecules, survival and growth factors (e.g. vascular endothelial growth factor [VEGF], angiogenin, plateletderived growth factor-B, transforming growth factor-B, and insulin-like growth factor-II), enzymes, proteins involved in tumor invasiveness (e.g., urokinases-type plasminogen activator), chaperones, nuclear factor kB (NFkB) and other resistance-related proteins.

Anoxia/hypoxia-induced proteome changes in neoplastic and stroma cells may lead to the arrest or impairment of neoplastic growth through molecular mechanisms, resulting in cellular quiescence, differentiation, apoptosis and necrosis. Cells exposed to hypoxia are generally arrested at the G1/S-phase boundary.³⁹ Under anoxia, most cells are arrested immediately, regardless of their position in the cell cycle.

Studies on tumors of the uterine cervix have demonstrated that tumor hypoxia is independent of patient and tumor characteristics such as, patient age, menopausal status, and parity, International Federation of Gynecology and Obstetrics (FIGO) stage, clinical tumor size, histopathological and grade of malignancy. In fact, tumor oxygenation was the strongest independent prognostic factor.⁴⁰

Adequate levels of oxygen are essential to effectively generate adequate tumoricidal prooxidant EMOD levels and to kill a wide range of cancer cell types and tumor hypoxia can be a serious limiting factor in reducing the effectiveness of radiotherapy, some O_2 -dependent cytotoxic agents and photodynamic therapy.⁴¹

PROOXIDANT CHEMOTHERAPEUTIC AGENTS

Cancer therapy can be aimed at the cell cycle, which consists of four phases, i.e., the G_1 , S, G_2 , and M phases. Based on their specificity, chemotherapy drugs can be classified as cell-specific agents (effective during certain cell cycle phases) and cell-cycle non-specific (effective during all phases of the cell cycle). Based on their specific characteristics and nature of treatment, chemotherapeutic agents can be classified as alkylating agents, anti-

metabolites, anthracyclines, antitumor antibiotics, monoclonal antibodies, platinums, or plant alkaloids.

PROOXIDANT EMOD PRODUCTION BY CHEMOTHERAPEUTIC AGENTS

Many chemotherapeutic drugs have well-defined mechanisms of actions, including traditional alkylating agents and anthracycline antitumor antibiotics, which generate EMODs. Depending upon specifics of oxidation/reduction potentials, these EMODs are uniformly subject to transformation to altered compounds by antioxidants through the simple process of electron transfer.

Doxorubicin, arsenic-induced apoptosis and 2-Methoxyestradiol induced apoptosis

Antineoplastic therapy can be based on the cell cycle and/or it can be based on the involvement of electronically modified oxygen derivatives (EMODs), formerly called oxygen free radicals or reactive oxygen species. These prooxidant EMOD reactants induce apoptosis and appear to be essential as activators for removing or killing cells that have accumulated mutations. 2-Methoxyestradiol induces apoptosis in Ewing sarcoma cells through mitochondrial hydrogen peroxide production. ⁴²⁻⁴⁴ Daunorubicin and doxorubicin can undergo redox cycling and produce EMODs, which can have a variety of effects, including damage to cell membranes and DNA-damage. ⁴⁵

BLEOMYCIN AND DOXORUBICIN

Bleomycin and doxorubicin are two agents known to generate prooxidant oxygen species.⁴⁶ In reactions involving Fe(II) and oxygen, an "activated" bleomycin species is formed that damages DNA through free radical intermediates.⁴⁷ Superoxide and hydrogen peroxide can also react with Fe(II) or Fe(III) bleomycin, respectively, to produce the activated form of the drug. DNA damage from bleomycin and ionizing radiation is similar in both induction and repair.⁴⁸

TAMOXIFEN, DOXORUBICIN, MITOMYCIN C, ETOPOSIDE AND CISPLATIN

Many chemotherapeutic drugs, such as tamoxifen, doxorubicin, mitomycin C, etoposide and cisplatin are superoxide (EMOD) generating agents and induce oxidative stress and apoptosis.^{49,50}

ANTHRACYCLINES

Reduction in EMOD levels generated by chemotherapeutic

agents has the same effect as a reduction in dose. ⁵¹ NADPHflavin reductase, cytochrome p450 reductase and mitochondrial NADH reductase can all reduce anthracyclines to a semiquinone radical. ⁵² This semiquinone radical can donate its free electron to molecular oxygen to generate the superoxide radical (O[•]₂). ⁵² Like hydrogen peroxide (H₂O₂), O[•]₂ can generate hydroxyl radicals ([•]OH) upon interaction with metal ions. ⁵² This results in lipid peroxidation of plasma membranes, leading to a loss of mitochondrial inner membrane potential and consequent cytochrome c release and apoptosis. EMODs can also directly damage DNA through generation of strand breaks and oxidized nucleic bases such as guanine to 8hydroxyguanine, giving rise to G-T transversions. ⁵²

However, as a caution, free radical generation by anthracyclines is thought to be responsible for the cardiotoxicity that puts some limits on their therapeutic use. ^{53, 54}

ADDITIONAL PROOXIDANT EMOD APOPTOSIS INDUCING AGENTS

Ideal treatment should aim to selectively kill the cancer cells, without harming normal cells. Elegant regulation of prooxidant EMOD levels may be a means to this exalted goal.

Cancer therapy seeks to utilize the sensitivity of transformed cells towards apoptotic signals, which allows the execution of apoptotic cell death.^{55, 56} Contrary to Harman's free radical theory in which EMODs are only deleterious, EMODs have been found to play a crucial beneficial role in intracellular apoptotic execution (cellular suicide).⁵⁷⁻⁶¹

GLIOMA PATHOGENESIS-RELATED PROTEIN 1 (GLIPR1), A P53 TARGET GENE

Glioma pathogenesis-related protein 1 (GLIPR1), a novel p53 target gene, is down-regulated by methylation in prostate cancer and has p53-dependent and -independent proapoptotic properties in tumorous cells. Investigators reported that the expression of GLIPR1 is significantly reduced in human prostate tumor tissues compared with adjacent normal prostate tissues and in multiple human cancer cell lines and that overexpression of GLIPR1 in cancer cells leads to suppression of colony growth and induction of apoptosis. Mechanistic analysis indicated that GLIPR1 up-regulation increases EMOD production leading to apoptosis through activation of the c-Jun–NH₂ kinase (JNK) signaling cascade. These results identify GLIPR1 as a

proapoptotic tumor suppressor acting through EMODs and the ROS-JNK pathway and support the therapeutic potential for this protein. 62

ELESCLOMOL (FORMERLY STA-4783)

Elesciomol (formerly STA-4783) is a novel small molecule undergoing clinical evaluation in a pivotal phase III melanoma trial (SYMMETRY). In a phase II randomized, double-blinded, controlled, multi-center trial in 81 patients with stage IV metastatic melanoma, treatment with elesclomol plus paclitaxel showed a statistically significant doubling of progression-free survival time compared with treatment with paclitaxel alone. Elesclomol induces apoptosis in cancer cells through the induction of oxidative stress (EMOD generation). Treatment of cancer cells in vitro with elesclomol resulted in the rapid generation of EMODs and the induction of a transcriptional gene profile characteristic of an oxidative stress response. Inhibition of oxidative stress by the antioxidant N-acetylcysteine (NAC) blocked the induction of gene transcription by elesclomol. In addition, N-acetylcysteine blocked drug-induced apoptosis, indicating that EMOD generation is the primary mechanism responsible for the proapoptotic activity of elesclomol. Excessive EMOD production and elevated levels of oxidative stress is believed by some to cause critical biochemical alterations that contribute to cancer cell growth. Thus, the induction of oxidative stress by elesclomol exploits this unique characteristic of cancer cells by increasing EMOD levels beyond a threshold that triggers cell death. 63

IMEXON

The antitumor agent imexon activates oxidative stress and antioxidant gene expression, which is evidence for EMOD production. Results show that a predominant biological effect of imexon is a change in redox state that can be detected in surrogate normal tissues as increased redoxsensitive transcription factor binding, EMOD generation and increased antioxidant gene expression.⁶⁴

CHAETOCIN

Investigators found that Chaetocin, a thiodioxopiperazine natural product previously unreported to have anticancer effects, was found to have potent antimyeloma activity in IL-6–dependent and –independent myeloma cell lines in freshly collected sorted and unsorted patient CD138⁺ myeloma cells and in vivo. Chaetocin displays superior ex vivo antimyeloma activity and selectivity than does doxorubicin and dexamethasone, and dexamethasone- or doxorubicin-resistant myeloma cell lines are largely non–cross-resistant to chaetocin. Mechanistically, chaetocin is dramatically accumulated in cancer cells via a process inhibited by glutathione and requiring intact/unreduced disulfides for uptake. Its anticancer (antimyeloma) in vitro and in vivo activity appears to be mediated primarily via the imposition of oxidative stress (prooxidant EMODs) and consequent apoptosis induction.⁶⁵

PCI-24781 (HISTONE DEACETYLASE [HDAC] INHIB)

Investigators examined the cytotoxicity and mechanisms of cell death of the broad-spectrum histone deacetylase (HDAC) inhibitor PCI-24781, alone and combined with bortezomib in Hodgkin lymphoma and non-Hodgkin lymphoma cell lines and primary lymphoproliferative (CLL/SLL) cells. PCI-24781 resulted in increased EMODs, oxidative stress and NF- B inhibition, leading to caspasedependent apoptosis. They showed that bortezomib is synergistic with PCI-24781. This combination or PCI-24781 alone has potential therapeutic value in lymphoma.⁶⁶

ZINC

Zinc is becoming increasingly important in regulating cancer cell growth and proliferation. Investigators showed that the anticancer agent motexafin gadolinium (MGd) disrupted zinc metabolism in A549 lung cancer cells, leading, in the presence of exogenous zinc, to cell death. They reported the effect of MGd and exogenous zinc on intracellular levels of free zinc, oxidative stress, proliferation, and cell death in exponential phase human B-cell lymphoma and other hematologic cell lines. They found that increased levels of oxidative stress, EMOD production and intracellular free zinc precede and correlate with cell cycle arrest and apoptosis.⁶⁷

QUINONES

Many naturally occurring quinones can be isolated from biological tissues. ⁶⁸ Also, chemotherapeutic drugs (adriamycin, daunorubicin, and mitomycin), acetaminophen (Tylenol), and air pollutants (cigarette smoke and automobile exhaust) are common source of quinones. Some quinones have potential to markedly induce the generation of prooxidant EMODs and may serve as the molecular mechanism of quinone cytotoxicity. ⁶⁸

RADIATION THERAPY

Hypoxic cancer cells are radio-resistant, which contributes dramatically to the inability of radiotherapy to control neoplastic growth and metastasis. Methods or therapies that provide increased prooxidant oxygen to cancer cells help radiation work more effectively by enabling more EMOD or free-radical formation. Radiation kills cancer cells by concentrating massive amounts of prooxidant free radicals directly into tumors.

Ionized radiation releases reactive oxygen species, i.e., EMODs, from the water molecule.⁶⁹ Thus cancer patients who undergo radiation therapy may be exposed to significant quantities of reactive prooxidant species. This may produce overkill or generate dangerously high prooxidant levels in areas outside of the treatment target site. Radiotherapy aims to alter cellular homeostasis, modify signal transduction pathways, alter redox states and induce cellular apoptosis. Exposure to ionizing radiation produces prooxidant oxygenderived free radicals including hydroxyl radicals (the most damaging), superoxide anion radicals, hydrogen peroxide and other oxidants.⁷⁰

And finally, as reported on 2-04-09 in the journal Nature, Stanford researcher, Robert Cho, found that breast cancer stem cells make much higher levels of protective antioxidants than other cancer cells. Use of a drug to block the antioxidant, glutathione, caused the cancer stem cells to become far more vulnerable to radiation. Using cells from mice and human breast cancer, the antioxidant glutathione protected the cancer cells from being killed by radiation EMOD-induced apoptosis.

However, even though EMODs are effective in killing tumor cells, they may threaten the integrity and survival of surrounding normal cells, which is dependent upon inherent tissue sensitivity and repair. Yet, the bottom line is that oxygen and its prooxidant EMOD agents are usually essential for effective radiation therapy and the induction of either apoptosis and/or necrosis.

HYDROGEN PEROXIDE THERAPY

Hydrogen peroxide appears to have medical attributes but has received little support in modern medicine. Hydrogen peroxide (H_2O_2) is a moderate oxidant that induces apoptosis of tumor cells in vitro.⁷¹

Even though the Baylor group's research on cancer, heart disease, wound healing and infections in the 1960s on

hydrogen peroxide was ground breaking, it has remained in obscurity. Still, it teaches the therapeutic potential of hydrogen peroxide in the treatment of cancer, wound healing, atherosclerosis, shock management and infectious diseases. Peroxide has been used widely in Europe and has had an impressive record of safety and effectiveness.

Many clinical and experimental applications of hydrogen peroxide have been demonstrated. In over 300 patients regional intra-arterial hydrogen peroxide potentiated the effect of radiation therapy for malignancy involving the head, neck, pelvis and retro-peritoneum.⁷² Increased localization of radioactive isotopes in malignant tumors was achieved by regional and intra-arterial infusion of hydrogen peroxide.^{73, 74} Oxygen enhanced environments were shown to be bactericidal for most clostridia species and inhibited alpha toxin release. Hyperbaric oxygen was shown to be a beneficial adjunct to therapy in Bacteroides fragilis, Fusobacterium infections and nonclostridial anaerobic soft tissue infections.⁷⁵

Results with hyperbaric oxygen are similar to that obtained by the Baylor investigators using intra-arterial and intravenous $\rm H_2O_2$.

Hydrogen peroxide appears to have two distinct effects. It initially inhibits the caspases and delays apoptosis. Then, depending on the degree of the initial oxidative stress, the caspases are activated and the cells die by apoptosis, or they remain inactive and necrosis occurs.^{76, 77} Some investigators believe that AIDS and cancer can be helped with hydrogen peroxide because of its induction of interferon-gamma production and its interactions which can produce a wide variety of oxygen derivatives.⁷⁸

In a simple but rather elegant experiment, Davies showed that cellular division or cell death is EMOD concentration dependent, when utilizing the EMOD, H_2O_2 . Cellular responses go from proliferation, to arrest, to apoptosis.⁷⁷ Those opposing hydrogen peroxide use have accused it of acting as a "genotoxicant or epigenetic" agent but although H_2O_2 can cause DNA damage, it is, at best, a very weak mutagen in mammalian cells.⁷⁹

INTRAVENOUS VITAMIN C MEGADOSES AND HYDROGEN PEROXIDE

Vitamin C (ascorbate, ascorbic acid) has had a controversial history in the prevention of cancer. Based on the pioneering work of Dr. Hugh Riordan, there have been some significant

subsequent developments. One clinical case report by Drisko et al showed that vitamin C together with other oxidants, when added adjunctively to first-line chemotherapy, prevented recurrence in two ovarian cancer patients.⁸⁰ This high dose, intravenous vitamin C therapy was believed to operate through the generation of hydrogen peroxide. Ascorbate-mediated cell death was due to protein-dependent extracellular H_2O_2 generation (i.e., prooxidant EMOD generation). Ascorbate, an electron-donor in such reactions, ironically initiates prooxidant chemistry and H_2O_2 formation. It was concluded that ascorbate at pharmacologic concentrations in blood is a pro-drug for H_2O_2 delivery to tissues.^{81,82}

Vitamin C acts as a cosubstrate for hydroxylase and oxygenase enzymes for the biosynthesis of procollagen, carnitine, and neurotransmitters.⁸³ These enzymes produce EMODs and ascorbate acts as a cosubstrate for them and thus, acts as a prooxidant.⁸⁴ Chen et al showed that at pharmacologic concentrations, ascorbate acts as a prooxidant, hydrogen peroxide generating agent, which exhibits selective cytotoxicity towards a wide variety of cancer cells in vitro and in vivo.^{85, 86} Even though there is much to be discovered in the ascorbate and hydrogen peroxide system, this appears to be an area of great potential.

Yet, in contrast, several vitamin C and iron cosupplementation studies, both in animals and humans, indicate that vitamin C inhibits rather than promotes irondependent oxidative damage.⁸⁸

PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) holds considerable promise in treating cancer but current terminology leads to confusion.

First, we need a definition of terms:

Phototherapy - light, UV, etc., is shown on to the skin, such as treating hyperbilirubinemia in babies.

Photochemotherapy - uses a photosensitizer like, psoralin

Photodynamic therapy - uses a photosensitizer given to the patient to produce ${}^{1}O_{2}*$ (excited singlet oxygen).

Photo-oxidative therapy - also referred to as photo irradiative therapy, uses UV light shown on blood which is returned to the body.

Bio-oxidative therapy - aerobic exercise.

Autohemotherapy - ozone.

Photodynamic effect - a photon is absorbed by a photosensitizer and raises it to its lowest triplet excited state, it diffuses until it collides with O_2 and raises it to its lowest singlet state.

Photodynamic therapy requires a photosensitive compound and a light source (usually a laser) capable of energizing electrons to higher orbitals (excited states). These excited molecules in turn excite triplet oxygen to one of its singlet excited states in accordance with the amount of energy transferred to oxygen's outer orbital electrons.

The unique property of photosensitizers to selectively accumulate in malignant and dysplastic tissues is exploited in the treatment of malignancies. PDT can selectively destroy tumors with this simple concept. Compared to surgery and conventional thermal Yag and argon laser treatment, there is much less damage and disruption of the underlying and adjacent normal tissue structures with photodynamic therapy, since there is essentially no thermal damage to the tissues. Superficial treatments do not require sterile theater conditions and can be delivered in an outpatient setting. There is little post-treatment discomfort and the only significant side effect is residual photosensitivity.

Availability of ground state oxygen within the tumor can dramatically influence and limit direct tumor cell kill.⁸⁹ Photodynamic therapy (PDT) is a novel therapeutic method for the treatment of malignant tumors, which utilizes prooxidant EMOD generation and in particular metastable singlet oxygen ($^{1}O_{2}*$). By combining PDT with hyperoxygenation, any underlying hypoxic condition is improved and the cell killing rate at various time points after PDT is dramatically enhanced.^{90,91}

In 1991, investigators described an apoptotic response to PDT. ⁹² Prooxidant species, especially singlet oxygen, produced by photosensitization or derived from cytotoxic agents, can activate apoptotic pathways. ⁹³ However, malignant cell types can exhibit an impaired ability to undergo apoptosis. PDT-mediated oxidative stress induces a transient increase in the downstream early response genes c-fos, c-jun, c-myc, and egr-1.⁹⁴

The in vivo tumoricidal reaction after PDT is accompanied by a complex immune response. PDT is a highly effective means of generating tumor-sensitized immune cells that can be recovered from lymphoid sites distant to the treated tumor at protracted time intervals after PDT, which asserts their immune memory character.^{95,96} Vascular shutdown is clearly an important aspect of PDT.⁹⁷

Clearly, when generated under carefully controlled conditions using exogenous sensitizers and light in the visible range (400 -700 nm), ${}^{1}O_{2}*$ can be exploited for therapeutic purposes, as in antineoplastic photodynamic therapy (PDT). In biological systems, singlet oxygen has a short lifetime of <0.04 ls and has also been shown to have a short radius of action of <0.02 lm.⁹⁸

However, in a cell with quenchers or scavengers abounding, ${}^{1}O_{2}*$ lifetime can be <50 nsec with a diffusion distance <10 nm from its point of origin, which is less than 0.1% of the radius of an average eukaryotic cell. This short distance of reactivity can have clinical and therapeutic benefits and limit the target area or "zone of reactivity."

Although controversial, it is important to remember that all antibodies apparently go through a singlet oxygen and ozone step. Antibodies can generate hydrogen peroxide (H_2O_2) from singlet molecular oxygen (1O_2*). This process is catalytic, and investigators identified the electron source for a quasi-unlimited generation of H_2O_2 . Antibodies produce up to 500 mole equivalents of H_2O_2 from 1O_2* , without a reduction in rate. This work shows the enormous potential for H_2O_2 production by antibodies and their prooxidant mechanism of action.^{99, 100}

THE HOWES SINGLET OXYGEN (O*) CANCER THERAPY SYSTEM

Howes proposed a singlet oxygen generating system composed of physiological agents for the eradication of cancer, which did not have the limitations of conventional photodynamic therapy, radiation therapy or chemotherapeutic systems. In a pilot study at Tuft's Medical School, athymic mice, which had received human squamous cell carcinoma, experienced a 22.7% tumor disappearance rate in the "high dose group" following injection with the Howes singlet oxygen producing system.¹⁰¹

Even more encouraging results were seen, with an initial 80% disappearance rate, when basal cell skin cancers were similarly injected with this singlet oxygen delivery system.

PDT generates similar products, in particular ${}^{1}O_{2}*$, with similar chemical reactivity as the Howes Singlet Oxygen

Delivery system.

COMMONALITY BETWEEN PDT AND THE HOWES SINGLET OXYGEN THERAPY SYSTEM

Pioneering work in the 1970s by Howes and Steele on microsomal lipid peroxidation ¹⁰³ and aryl-hydroxylations ¹⁰⁴ demonstrated evidence for the generation and participation of electronic excitation states, namely singlet oxygen. This was the first demonstration of a functional generation of an electronic excitation state, exclusive of vision, in mammalian systems. Their proposal, that singlet oxygen is the identity of the long sought out "active oxygen" acting on the cytochrome P 450 microsomal mixed function oxidases, has more recently been supported by the work of Yasui et al in 2002. ¹⁰⁵

While studying widely divergent biological electronic excitation generating systems, such as the microsomal mixed function oxidases, the neutrophil respiratory burst ¹⁰⁶ and proline hydroxylation for collagen biosynthesis, one of the investigators (Howes) believed that these oxidative systems shared a point of convergence, expressed in the Howes Excytomer Pathway, involving superoxide anion and electronically excited singlet oxygen. ¹⁰⁷

Furthermore, Howes saw an additional commonality with generation of singlet oxygen produced by the steady-state physiological oxidative reagents containing an organic peroxide and the salt of hypohalous acid ¹⁰⁸

Subsequently, Howes reasoned that the

peroxide/hypochlorite oxidative system may represent an ideal method of singlet oxygen delivery for effectively treating premalignant and malignant lesions, while simultaneously eliminating many of the drawbacks associated, not only with PDT, but with all other conventional methods of cancer therapy, including chemotherapy and irradiation. The peroxide/hypochlorite oxidative system has been shown to generate primarily singlet oxygen exclusively, as opposed to hydroperoxide/hypochlorite systems which have been shown to produce peroxyl and alkoxyl radicals.¹⁰⁹

OZONE THERAPY

Ozone therapy is practiced in most mainland European countries and the recently passed Alternative Therapy Legislation has made ozone therapy an option for patients in the USA in Alaska, Arizona, Colorado, Georgia, Minnesota, New York, New Jersey, North Carolina, Ohio, Oklahoma, Oregon, South Carolina, and Washington. Ozone therapy is not prohibited in Bulgaria, Cuba, Czech Republic, France, Germany, Greece, Israel, Italy, Japan, Malaysia, Mexico, Poland, Romania, Russia, Switzerland, Turkey, United Arab Emirates and Ukraine. Still, it remains on the fringe of mainstream medicine in America and the American Cancer Foundation has always strongly advised cancer patients against ozone therapy, as it does for other "Questionable methods of cancer management: hydrogen peroxide and other 'hyperoxygenation' therapies." ¹¹⁰

However, scientific studies have found support for ozone therapy and investigators at Washington University discovered ozone inhibited growth of lung, breast and uterine cancer cells in a dose dependent manner while healthy tissues were not damaged by ozone.¹¹¹

French studies have shown that ozone enhanced the treatment of chemo-resistant tumors and acted adjunctively with 5-fluorouracil chemotherapy in tumors derived from the colon and breast.¹¹²

Research has shown that ozone therapy can improve oxygenation in hypoxic tumors.¹¹³⁻¹¹⁵ A 2004 study at Oxford University, using a human trial of ozone therapy, involving 19 patients with incurable head and neck tumors receiving radiotherapy and tegafur, plus either chemotherapy or ozone therapy, concluded that results warrant further research of ozone as a treatment for cancer.¹¹⁶

Cuban studies in rats ^{117, 118} and Russian human trials report benefits of complimentary ozone treatment and as regards drug complications. ¹¹⁹⁻¹²¹

A 2008 study by Schulz et al, published in the International Journal of Cancer, found that survival of NewZealand White rabbits with head and neck squamous cell carcinoma could be enhanced by peritoneal insufflation of a medical ozone/oxygen gas mixture.

HYPERBARIC OXYGEN THERAPY

It has been reported that hyperbaric oxygen therapy, using pressures at or less than 2.5 ATA, do not significantly increase EMODs in the presence of normal antioxidant defenses. Hyperbaric oxygen increases the oxygen in tumor tissue, as well as EMOD and prooxidant levels, and appears to enhance the efficiency of PDT.^{122, 123} Hyperoxygenation appears to provide effective ways for improving PDT efficiency by oxygenating both preexisting and treatment-induced cell hypoxia.¹²⁴

RELEVANT GENERAL INFORMATION

Lest we forget, oxygen and prooxidant EMODs play a central protective role against pathogens, as well as a crucial role in cancer therapy.

Polymorphonuclear cells (PMNs) require oxygen to kill organism by producing prooxidant superoxide, hydrogen peroxide, singlet oxygen and other products via the respiratory burst.¹²⁵ The PMN is protected by detoxifying free radicals with superoxide dismutase, catalase and glutathione. It has been shown in numerous studies that the degree of polymorphonuclear cell function in killing of bacteria is directly dependent on oxygen tension.^{126, 127}

Scientists at The Ohio State University (OSU) have identified a way to predict very early in the treatment process the outcome of radiation and chemotherapy for cervical cancer patients and it is based on oxygen levels within the tumor. According to Jian Z. Wang, the oxygenation of a tumor is critical for the success of cancer treatment because the amount of oxygen in a cell is directly correlated with the ability of that cell to repair radiation damage. Wang stated that, "Inevitably, those welloxygenated tumor cells die, tumors are less likely to return, and patient survival rates rise." The research was described in the talk, "When the Oxygen Level Matters Mostly During Radiation Therapy of Cervical Cancer?" presented July 31, 2008 at the 50th annual meeting of the American Association of Physicists in Medicine.

Men with a low oxygen supply to their prostate tumor have a higher chance of the prostate cancer returning, as found by increasing prostate-specific antigen (PSA) levels following treatment, according to Benjamin Movsas, M.D., senior study author and chair of the Department of Radiation Oncology at Henry Ford Hospital. Moreover, recent studies suggest the same finding also appears to apply to patients treated with surgery. Movsas stated that "A tumor's oxygen supply can significantly predict outcome following treatment, independent of tumor stage or Gleason score (a classification of the grade of prostate cancer)." ¹²⁸

In short, consideration of oxygen levels in cancer chemotherapy is crucial for successful eradication of neoplasia.

Various cancer chemopreventive agents can induce apoptosis in premalignant and malignant cells in vivo and/or in vitro, which serve as an anticancer mechanism. Many of these apoptogenic-inducing agents function as prooxidants in vitro.

Significant in vitro data exists showing that antioxidants can block EMOD-induced apoptosis for a wide variety of cancerous cell types, such as leukemia, lymphoma, retinoblastoma, myeloma, pheochromocytoma and human cancers of the breast, lung, pancreas, liver, colon, rectum and endometrium. This data can not be ignored when considering effective prooxidant cancer therapy.¹²⁹

In 2001, Harvard Medical School investigators observed a dose dependent inhibition of MBT-2 cell (murine bladder cancer) growth after exposure to doxorubicin hydrochloride, which could be enhanced by hydrogen peroxide and inhibited by preincubation with alpha tocopherol. They concluded that hydrogen peroxide may be a relatively inexpensive, nontoxic method of augmenting the cytotoxicity of doxorubicin hydrochloride.¹³⁰

To avoid the confusion with terms of the past, it is suggested that current scientific oxygen related therapies should be referred to as "prooxidant EMOD therapies."

CONCLUSION

The salutary role of EMODs in oncologic therapy has been scientifically substantiated by the use of prooxidant EMODs in currently available anti-cancer therapeutic methods, such as chemotherapy, radiation, photodynamic therapy, etc. Points of confluence exist within the many cancer methods available to treat cancer and many share the interaction of prooxidants. It also suggests potential courses of action clinicians may take when patients express an interest in prooxidant therapies or combinations thereof. Prooxidant EMODs have been proven to exhibit tumoricidal activity in both in vitro and in vivo studies. We must move forward and beyond the outdated and negative history surrounding socalled "oxidative therapies." Many prooxidant agents suggest selectivity in promoting the death of cancerous cells and avoidance of harm to normal cells. The prooxidant approach to cancer therapy begs for further scientific inquiry and additional validation.

Contrary to the unsupported and irresponsible statements of some major cancer agencies, this review clearly demonstrates that some prooxidant EMODs (a.k.a. oxidative therapies) are currently and have been for decades, integral, effective and safe theoretical and clinical agents in the battle against cancer. To deny the scientific facts supporting prooxidant EMOD therapies is to deny patients significant

treatment modalities, which may be crucial to their survival. Various medically related organizations may deny the truth

surrounding prooxidant cancer therapy but they can not

change the truth, which is exposed to all by a review of the literature.

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