

Free Radicals: The Pros and Cons of Antioxidants

Cancer Chemotherapy and Antioxidants¹

Kenneth A. Conklin²

Jonsson Comprehensive Cancer Center, UCLA, Los Angeles, CA 90095-1778

EXPANDED ABSTRACT

KEY WORDS: • antioxidants • caspase • chemotherapy • cisplatin • coenzyme Q10 • death receptor • doxorubicin

Does the administration of antioxidants during cancer chemotherapy affect antineoplastic efficacy or the development of side effects? Although the majority of preclinical studies involving the use of in vitro systems and animal models support the contention that certain antioxidants are of benefit, few clinical studies have been done (1,2). Factors to consider in the design of studies to answer the question posed above include the properties of the individual antioxidants, the mechanism of action of the antineoplastic agents, and the mechanism whereby antineoplastic agents cause their side effects. Additionally, the impact of chemotherapy-induced oxidative stress upon antineoplastic efficacy and the role that reactive oxygen species (ROS)³ may play in drug-induced apoptosis need to be elucidated.

All antioxidants cannot be viewed as equal when evaluating their potential impact on cancer chemotherapy, and an individual antioxidant cannot be anticipated to have the same impact on the activity of all cancer chemotherapeutic agents. Small molecular weight antioxidant molecules are effective reducing agents but some, including glutathione (GSH), N-acetyl cysteine (NAC), and alpha-lipoic acid, also are strong nucleophiles because they possess a sulfhydryl group. While all antioxidants are capable of detoxifying free radicals, those that possess strong nucleophilic properties can bind and inactivate the electrophilic intermediates of antineoplastic agents that act via nucleophilic substitution reactions, i.e., platinum-coordination complexes and most alkylating agents. Competition between nucleophilic antioxidants and the nucleophilic cellular targets of these anticancer agents can reduce the

efficacy of the therapy. Selenium also can be considered a nucleophilic antioxidant. Although inorganic selenium does not function as an antioxidant, it is incorporated into selenoproteins (as selenocysteine or selenomethionine) and other selenium compounds such as methylselenol. Because selenium possesses properties similar to sulfur, selenoproteins and organoselenols have nucleophilic properties. Additionally, selenium induces the synthesis of the cysteine-rich metallothioneins, which can bind to electrophilic intermediates of platinum-coordination complexes and alkylating agents, and elevated levels of metallothioneins are associated with resistance to these antineoplastic agents (3).

If generation of ROS by a cancer chemotherapeutic agent or a free radical intermediate of the drug plays a role in its cytotoxicity, antioxidants may interfere with the drug's antineoplastic activity. However, if the reactive species are responsible only for the drug's adverse effects, antioxidants may actually reduce the severity of such effects without interfering with the drug's antineoplastic activity. Thus, it is important to distinguish between a drug's ability to induce oxidative stress in biological systems and the role, if any, that ROS or free radical intermediates play in the mechanism of action of the drug.

The drugs of many classes of antineoplastic agents are known to generate a high level of oxidative stress in biological systems (1). These classes of drugs include the anthracyclines, most alkylating agents, platinum-coordination complexes, epipodophyllotoxins, and camptothecins. For these drugs, the hepatic microsomal monooxygenase system is a primary site where ROS are generated, although other enzymatic (e.g., xanthine oxidase) and nonenzymatic (Fenton and Haber-Weiss reactions) mechanisms also play a role. The electron transport system of cardiac mitochondria is another site where significant levels of ROS are generated by anthracyclines (4). Although some classes of antineoplastic agents generate high levels of oxidative stress, others, including the taxanes, vinca alkaloids, antifolates, and nucleoside and nucleotide analogues, generate only low levels. Nevertheless, all drugs generate some free radicals as they induce apoptosis in cancer cells.

One of the primary pathways of drug-induced apoptosis is the pathway that involves release of cytochrome c from mito-

¹ Presented as part of the conference "Free Radicals: The Pros and Cons of Antioxidants," held June 26–27 in Bethesda, MD. This conference was sponsored by the Division of Cancer Prevention (DCP) and the Division of Cancer Treatment and Diagnosis, National Cancer Institute, NIH, Department of Health and Human Services (DHHS); the National Center for Complementary and Alternative Medicine (NCCAM), NIH, DHHS; the Office of Dietary Supplements (ODS), NIH, DHHS; the American Society for Nutritional Science; and the American Institute for Cancer Research and supported by the DCP, NCCAM, and ODS. Guest editors for the supplement publication were Harold E. Seifried, National Cancer Institute, NIH; Barbara Sorkin, NCCAM, NIH; and Rebecca Costello, ODS, NIH.

² To whom correspondence should be addressed.
E-mail: kconklin@mednet.ucla.edu.

³ Abbreviations used: GGT, γ -glutamyl transpeptidase; GSH, glutathione; NAC, N-acetyl cysteine; ROS, reactive oxygen species.

chondria (5). When cytochrome c is displaced from the electron transport chain, instead of electrons being transferred to oxygen via cytochrome c oxidase with the formation of water, electrons are diverted from NADH dehydrogenase (Complex I) and reduced coenzyme Q₁₀ to oxygen with the concomitant formation of superoxide radicals. Although superoxide is not highly toxic, mitochondrial superoxide dismutase generates hydrogen peroxide from superoxide and, in the presence of reduced iron that is abundant in mitochondria, highly toxic hydroxyl radicals are formed via Fenton and Haber-Weiss reactions. Thus, all drugs that induce apoptosis by this mechanism generate some degree of oxidative stress, although this does not imply that free radical generation is necessary for a drug to exert its cytotoxic effect on neoplastic cells, because the apoptotic process is initiated by cytochrome c release and superoxide generation occurs secondarily.

The mechanism of action of only a few antineoplastic agents has been definitively linked to a free radical intermediate of the parent drug. Examples of antineoplastic agents that have been so linked include mitomycin-C, which creates DNA interstrand crosslinks following reduction of its aziridine ring, and bleomycin, which cleaves DNA by hydrogen abstraction by its iron-binding arm, which functions as a ferrous oxidase. Most of the other major classes of antineoplastic agents have well-established mechanisms of action that are independent of free radical intermediates or free radical generation. These include the antifolates and nucleoside and nucleotide analogues that impact DNA synthesis, the vinca alkaloids and taxanes that interfere with microtubule function, the epipodophyllotoxins (etoposide, teniposide) that interfere with topoisomerase II activity, and the camptothecins (topotecan, irinotecan) that interfere with topoisomerase I activity.

The platinum coordination complexes (cisplatin, carboplatin, oxaliplatin) and most alkylating agents form strong electrophilic intermediates that act via nucleophilic substitution reactions to form inter- and intrastrand DNA crosslinks. Although toxicity among these agents varies, most side effects also are attributed to nucleophilic substitution reactions, e.g., cisplatin toxicity (nephrotoxicity, neurotoxicity, ototoxicity) is attributable to protein sulfhydryl binding and inactivation of thiol-containing enzymes. Antioxidants that act as reducing agents do not appear to interfere with the antineoplastic activity of these agents nor do they prevent the development of side effects (1), which suggests that free radical generation does not play a role in the antineoplastic activity or the toxicity of these agents. In contrast to antioxidants that act as reducing agents, nucleophiles, such as GSH, NAC, and thiosulfate, can bond covalently to electrophilic compounds such as cisplatin, and mixing cisplatin with thiosulfate prior to administration blocks the antineoplastic agent's activity (1). However, several animal and clinical studies have shown that intravenous administration of GSH shortly before administration of cisplatin reduces the drug's toxicity without reducing its anticancer activity (1). This may be explained by the high renal and neural intracellular levels of γ -glutamyl transpeptidase (GGT), an enzyme that hydrolyzes circulating GSH (GluCysGly) to Glu and CysGly and transports these products into the cell (6). The high enzyme levels in normal tissues allow for rapid clearing of circulating GSH, thus preventing cisplatin binding to GSH in the bloodstream, and also results in high GSH levels in renal and neural tissue, thus protecting them from cisplatin toxicity. Renal cells also have unique mechanisms for concentrating selenium and for formation of methylselenol and glutathionylselenol, compounds that also protect the kidneys from cisplatin toxicity (7). Most cancer cells have low levels of GGT. Thus, intravenous administra-

tion of GSH does not increase the GSH content of most cancer cells that could reduce the antineoplastic activity of cisplatin. However, GTT may be expressed in higher amounts or be inducible in some neoplastic cells (8). The potential selective protection of normal tissues from cisplatin toxicity warrants further investigation.

Several mechanisms have been proposed for the anticancer activity of anthracyclines. Although the most studied anthracycline, doxorubicin, alters membrane function, signal transduction such as pathways involving protein kinase C, and many other cellular functions, the most compelling evidence for its primary mechanism of action is via intercalation with double-stranded DNA and inhibition of topoisomerase II activity. This effect is evident at clinically relevant concentrations, with the drug being localized primarily in the nucleus of neoplastic cells and acting in the S-phase of the cell cycle. This supports topoisomerase II inhibition as the drug's primary cytotoxic mechanism. However, doxorubicin readily undergoes a one-electron reduction to its semiquinone, which can donate an electron to molecular oxygen resulting in superoxide generation. Although generation of hydroxyl radicals from superoxide is an attractive explanation for the cytotoxicity of doxorubicin, several lines of evidence suggest that this mechanism does not contribute significantly to the drug's anticancer activity: (a) doxorubicin is localized primarily in the nucleus of neoplastic cells, and DNA-intercalated doxorubicin is not readily reduced; (b) although semiquinone formation can occur in membranes, including the nuclear membrane and the sarcoplasmic reticulum, the hepatic microsomal cytochrome P450 monooxygenase system and mitochondria of cardiac cells are the primary sites where high levels of the semiquinone are generated; (c) preclinical studies show that antioxidants reduce doxorubicin-induced oxidative stress while preserving the drug's antineoplastic activity (1,2); (d) several clinical studies suggest that antioxidants such as coenzyme Q₁₀ do not interfere with the drug's anticancer efficacy (1,2); (e) most studies that have shown doxorubicin-induced hydroxyl radical formation to be cytotoxic have used concentrations (several micromolar) that are far above those that are clinically relevant (a 60 mg/m² bolus dose results in a peak level of 1 micromolar doxorubicin, which rapidly declines to a level of ~10 nanomolar, which is sustained for a period of up to 1 wk); (f) doxorubicin has been shown to be cytotoxic under hypoxic conditions; and (g) many neoplastic cells are not sensitized to doxorubicin by depletion of antioxidants.

Although free radical generation may not play a significant role in the antineoplastic activity of doxorubicin, there is compelling evidence that disruption of the electron transport system and hydroxyl radical generation in mitochondria of cardiac cells accounts for the drug's acute and chronic cardiotoxicity. The selective toxicity of doxorubicin to cardiac cells is accounted for by the unique structure of the cardiac mitochondrial inner membrane, which possesses a cytosolic (outer surface, or intermembranous) NADH dehydrogenase in addition to the matrix (inner surface) NADH dehydrogenase that is present in the mitochondria of all cells (9). Doxorubicin (a tetracycline ring with a sugar moiety) is hydrophilic and cannot penetrate the inner membrane and be reduced by the matrix enzyme. However, in cardiac mitochondria, doxorubicin, which can penetrate the outer membrane and enter the mitochondrial cytosol, is reduced by the cytosolic NADH dehydrogenase to its semiquinone. Intramolecular rearrangement results in formation of the lipophilic deoxyglycone of doxorubicin that penetrates the inner membrane where it then inhibits coenzyme Q₁₀-dependent enzymes, accounting for disruption of mitochondrial energetics and resulting in the

development of acute cardiotoxicity (arrhythmias and reduced ejection fraction). The deoxyaglycone also competes with coenzyme Q_{10} (both structurally are quinones) as an electron acceptor, diverting electrons to molecular oxygen with the formation of superoxide radicals, and displaces coenzyme Q_{10} from the electron transport chain, resulting in elevated plasma levels of coenzyme Q_{10} (10). These effects explain the generation of elevated levels of ROS in cardiac cells that lasts for several weeks following administration of doxorubicin and the high levels of mitochondrial DNA adducts that form in heart mitochondria and result in suppression of mitochondrial gene expression for critical components of the electron transport system (11), such as coenzyme Q_{10} (12). These long-lasting effects most likely explain mitochondrial disruption, the first cytological evidence of chronic cardiotoxicity (13,14), which leads to myocyte degeneration and cardiac failure. Preclinical and a limited number of clinical studies suggest that, whereas antioxidants in general do not prevent doxorubicin cardiotoxicity, administration of coenzyme Q_{10} does prevent the development of both acute and chronic cardiotoxicity without interfering with the drug's anticancer efficacy (1). This is another area that warrants further investigation.

Oxidative stress induced by low levels of hydrogen peroxide has been shown to elevate the LD_{50} of several types of antineoplastic agents and to block drug-induced apoptosis in neoplastic cells, causing cells to undergo necrosis instead of apoptosis (15,16). These effects of hydrogen peroxide are prevented by the addition of certain antioxidants. The reduced cytotoxicity of anticancer agents in the presence of hydrogen peroxide, an effect that also might occur during chemotherapy-induced oxidative stress, may result from the effects of the cellular products generated by ROS.

Free radicals generated during oxidative stress have many cellular targets, but one of the primary targets is cellular lipids. Lipid peroxidation of PUFA results in formation of alkoxyl and peroxy radicals (primary products) that are highly reactive and relatively short-lived. Secondary products of lipid peroxidation include numerous aldehydes, including malondialdehyde, the 4-hydroxyalkenals, and acrolein (17). These electrophilic compounds are more stable than the primary products, and they can diffuse throughout the cell where they can damage cellular components and interfere with cellular functions. The aldehydes are potent enzyme inhibitors because they bind to nucleophilic groups of amino acids, such as cysteine, lysine, histidine, serine, and tyrosine, that are critical components of enzyme active sites or necessary for maintaining the tertiary structure of enzymes.

Oxidative stress, possibly through aldehyde-mediated enzyme inhibition of cyclin-dependent kinases, inhibits transition of cells from the G_0 phase (quiescent phase) of the cell cycle to the G_1 phase, blocks progression through the restriction point, and causes arrest of the cell cycle at the G_1 , S, G_2 , and M phase checkpoints (18). For antineoplastic agents that exhibit cell cycle phase-specific activity, e.g., those that interfere with DNA synthesis or block the mitotic process, interference with cell cycle progression may diminish their cytotoxicity. Even platinum coordination complexes and alkylating agents, which are not considered to be phase-specific agents, require cells to progress through the S phase and G_2 phase for apoptosis to occur. Checkpoint arrest also may allow for DNA repair of damage caused by platinum coordination complexes and alkylating agents, and checkpoint abrogation (the opposite of what happens during oxidative stress) has been shown to enhance the cytotoxicity of several types of antineoplastic agents. By reducing aldehyde generation, antioxidants may counteract the effects of chemother-

apy-induced oxidative stress on cell cycle progression and enhance the cytotoxicity of antineoplastic agents.

Aldehydes also may directly interfere with the major pathways of chemotherapy-induced apoptosis, namely, the CD95/CD95 ligand (Fas/Apo1) death receptor pathway and the pathway initiated by cytochrome c release from mitochondria (5). The CD95 death receptor has a cysteine-rich extracellular domain, making it a potential target for binding by strong electrophiles such as the aldehydes. Binding of aldehydes to death receptors may mimic the effect of death receptor antibodies that interfere with ligand binding and block drug-induced apoptosis.

Whereas oxidative stress can act as a trigger for cytochrome c release and initiate apoptosis, excessive oxidative stress is an effective inhibitor of caspases (and procaspase activation) (19,20), the enzymes that carry out the apoptotic process. Caspases are cysteine proteases, possessing a cysteine moiety at their active sites, and require a reducing environment for optimal activity. Caspase inhibition, such as that caused by the cowpox virus CrmA protein when it is overexpressed in leukemic cells, confers resistance to a variety of antineoplastic agents (21). Electrophilic aldehydes, such as acetyl-tetrapeptide (22), also bind to the active site of caspases and inhibit their activity. Thus, aldehyde generation, resulting in caspase inhibition, may account for the reduced efficacy of antineoplastic agents during oxidative stress. If so, antioxidants may enhance the anticancer activity of cancer chemotherapeutic agents by reducing aldehyde generation that is caused by chemotherapy-induced oxidative stress.

Future research needs to address many unanswered questions regarding the impact of oxidative stress on the therapeutic efficacy of cancer chemotherapy, the role that oxidative stress plays in the development of chemotherapy-induced side effects, and the effect of antioxidants on anticancer activity and the development of therapy-induced adverse effects. Fundamental studies that elucidate the impact of oxidative stress, and specifically ROS-generated aldehydes, on cell cycle progression and apoptotic pathways may guide us to interventions that could enhance chemotherapeutic efficacy. Further investigation of GSH for preventing cisplatin toxicity and coenzyme Q_{10} for preventing doxorubicin cardiotoxicity appears to be indicated based upon existing studies. Finally, clinical studies must be conducted to determine both the short-term and long-term impact of antioxidants, singly and in combination, upon the efficacy of cancer chemotherapy and the development of chemotherapy-induced side effects.

LITERATURE CITED

1. Conklin, K. A. (2000) Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects. *Nutr. Can.* 37: 1-18.
2. Lamson, D. W. & Brignall, M. S. (1999) Antioxidants in cancer therapy; their actions and interactions with oncologic therapies. *Altern. Med. Rev.* 4: 304-329.
3. Tobey, R. A., Enger, M. D., Griffith, J. K. & Hildebrand, C. E. (1982) Zinc-induced resistance to alkylating agent toxicity. *Can. Res.* 42: 2980-2984.
4. Gille, L. & Nohl, H. (1997) Analyses of the molecular mechanism of adriamycin-induced cardiotoxicity. *Free Rad. Biol. Med.* 23: 775-782.
5. Solary, E., Droin, N., Bettaieb, A., Corcos, L., Dimanche-Boitrel, M. T. & Garrido, C. (2000) Positive and negative regulation of apoptotic pathways by cytotoxic agents in hematological malignancies. *Leukemia* 14: 1833-1849.
6. Zunino, F., Pratesi, G., Micheloni, A., Cavalletti, E., Sala, F. & Tofanetti, O. (1989) Protective effect of reduced glutathione against cisplatin-induced renal and systemic toxicity and its influence on the therapeutic activity of the antitumor drug. *Chem. Biol. Interact.* 70: 89-101.
7. Baldwin, G. S., Mol, J.G.J., DeKanter, F.J.J., van Baar, B., de Goeij, J.J.M. & Vermeulen, N.P.E. (1991) The mechanism of interaction between cisplatin and selenite. *Biochem. Pharmacol.* 41: 1429-1437.
8. Daubeuf, S., Balin, D., Leroy, P. & Visvikis, A. (2003) Different mech-

anisms for gamma-glutamyltranspeptidase-dependent resistance to carboplatin and cisplatin. *Biochem. Pharmacol.* 66: 595–604.

9. Nohl, H. (1987) Demonstration of the existence of an organo-specific NADH dehydrogenase in heart mitochondria. *Eur. J. Biochem.* 169: 585–591.

10. Eaton, S., Skinner, R., Hale, J. P., Pourfarzam, M., Roberts, A., Price, L. & Bartlett, K. (2000) Plasma coenzyme Q₁₀ in children and adolescents undergoing doxorubicin therapy. *Clin. Chim. Acta.* 302: 1–9.

11. Zhou, S., Palmeira, C. M. & Wallace, K. B. (2001) Doxorubicin-induced persistent oxidative stress to cardiac myocytes. *Tox. Lett.* 121: 151–157.

12. Folkers, K., Liu, M., Watanabe, T. & Porter, T. H. (1977) Inhibition by adriamycin of the mitochondrial biosynthesis of coenzyme Q₁₀. *Biochem. Biophys. Res. Commun.* 77: 1536–1542.

13. Jaenke, R. S. (1974) An anthracycline antibiotic-induced cardiomyopathy in rabbits. *Lab. Invest.* 30: 292–304.

14. Domae, N., Sawada, H., Matsuyama, E., Konishi, T. & Uchino, H. (1981) Cardiomyopathy and other chronic toxic effects induced in rabbits by doxorubicin and possible prevention by coenzyme Q₁₀. *Can. Treat. Rep.* 65: 79–91.

15. Shacter, E., Williams, J. A., Hinson, R. M., Senturker, S. & Lee, Y. J. (2000) Oxidative stress interferes with cancer chemotherapy: inhibition of lymphoma cell apoptosis and phagocytosis. *Blood* 96: 307–313.

16. Lee, Y. J. & Shacter, E. (1999) Oxidative stress inhibits apoptosis in human lymphoma cells. *J. Biol. Chem.* 274: 19792–19798.

17. Esterbauer, H., Schaur, R. J. & Zollner, H. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Rad. Biol. Med.* 11: 81–128.

18. Conklin, K. A. (2002) Dietary polyunsaturated fatty acids: impact on cancer chemotherapy and radiation. *Altern. Med. Rev.* 7: 4–21.

19. Chandra, J., Samali, A. & Orrenius, S. (2000) Triggering and modulation of apoptosis by oxidative stress. *Free Rad. Biol. Med.* 29: 323–333.

20. Hampton, M. B. & Orrenius, S. (1997) Dual regulation of caspase activity by hydrogen peroxide: implications for apoptosis. *FEBS Lett.* 414: 552–556.

21. Antoku, K., Liu, Z. & Johnson, D. E. (1997) Inhibition of caspase proteases by CrmA enhances the resistance of human leukemic cells to multiple chemotherapeutic agents. *Leukemia* 11: 1665–1672.

22. Wilson, K. P., Black, J. F., Thomson, J. A., Kim, E. E., Griffith, J. P., Navia, M. A., Murcko, M. A., Chambers, S. P., Aldape, R. A., Raybuck, S. A. & Livingston, D. J. (1994) Structure and mechanism of interleukin-1beta converting enzyme. *Nature* 370: 270–275.