

Reversing mitochondrial dysfunction, fatigue and the adverse effects of chemotherapy of metastatic disease by Molecular Replacement Therapy

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Keywords: oxidative stress, alkylating agents, anthracyclines, doxorubicin, mitochondria, coenzyme Q₁₀, lipid peroxidation, electron transport chain, antioxidants, membranes

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*The authors have no financial interest in any products discussed in this contribution.

Running Title: Molecular Replacement in Therapy of Cancer

Abstract.

Metastatic cancers are associated with cellular oxidative stress, and during cancer chemotherapy excess drug-induced oxidative stress can limit therapeutic effectiveness and cause a number of side effects, including fatigue, nausea, vomiting, diarrhea and more serious adverse effects, such as cardiomyopathy, peripheral neuropathy, hepatotoxicity and pulmonary fibrosis. We review here the hypothesis that the acute and chronic adverse effects of cancer chemotherapy can be reduced by Molecular Replacement of membrane lipids and enzymatic cofactors, such as coenzyme Q₁₀. By administering nutritional supplements with replacement molecules and antioxidants, oxidative membrane damage and reductions of cofactors in normal tissues can be reversed, protecting and restoring mitochondrial and other cellular functions and reducing chemotherapy adverse effects. Recent clinical trials using cancer and non-cancer patients with chronic fatigue have shown the benefit of Molecular Replacement plus antioxidants in reducing the damage to mitochondrial membranes, restoring mitochondrial electron transport function, reducing fatigue and protecting cellular structures and enzymes from oxidative damage. Molecular Replacement and antioxidant administration mitigates the damage to normal tissues, such as cardiac tissue, and reduces the adverse effects of cancer therapy without reduction in therapeutic results.

Introduction

Oxidative stress is associated with cancer progression, aging and age-related degenerative diseases [1-3]. It is caused by an excess of reactive oxygen (ROS) and nitrogen (RNS) species over cellular antioxidants, resulting in oxidation of cellular structures, such as membrane lipids and proteins and mutation of mitochondrial and nuclear DNA [4-7]. ROS/RNS are naturally occurring cellular oxidants that are involved in gene expression, intracellular signaling, antimicrobial defense and other normal cellular processes, such as cell proliferation [8-10]. However, when ROS/RNS are in excess cellular damage can occur [4, 8, 10].

Under normal physiological conditions cellular antioxidant defenses maintain ROS/RNS at appropriate concentrations [11-13]. Endogenous cellular antioxidant defenses include the enzymes glutathione peroxidase, catalase, superoxide dismutase, among others [14, 15], and

low molecular weight dietary antioxidants [16,17]. Some of these dietary antioxidants have been used as natural chemopreventive agents to shift the balance of oxidative molecules towards more physiological levels [18, 19].

The promotion and progression of malignant cancers are linked to excess oxidative stress [24-26]. Oxidative stress and antioxidant status have been examined in various malignant cancers, such as breast [22-25], renal [26,27], prostate [28, 29], colorectal [30, 31], among other malignancies [32-34]. In all of these studies ROS/RNS were in excess of antioxidant properties, and thus these cancers were proposed to arise, in part, as a consequence of excess ROS/RNS and oxidative damage to the genetic apparatus [3, 4, 6, 7, 35].

Oxidative stress induced by chemotherapy

Chemotherapeutic agents cause the generation of excess ROS/RNS in biological systems [36, 37]. Thus, individuals receiving cytotoxic chemotherapy are exposed to excess oxidative stress. The highest levels of oxidative stress are generated by anthracycline antibiotics (e.g., doxorubicin, daunorubicin, and epirubicin), although alkylating agents, platinum-coordination complexes (e.g., cisplatin, carboplatin, and oxaliplatin), epipodophyllotoxins (e.g., etoposide and teniposide), and camptothecins (e.g., topotecan and irinotecan) can also produce high levels of ROS/RNS [36, 37].

The primary site of ROS/RNS generation is the cytochrome P450 monooxygenase system of hepatic microsomes [36, 38]. Enzyme systems such as the xanthine-xanthine oxidase system, and non-enzymatic mechanisms, such as Fenton and Haber-Weiss reactions, also play a role in creating excess oxidative stress during chemotherapy. The very high levels of oxidative stress generated by anthracyclines is due to their ability to displace coenzyme Q₁₀ (CoQ₁₀) from the electron transport system of cardiac mitochondria (see below), resulting in diversion of electrons directly to molecular oxygen with the formation of superoxide radicals [36-38].

Some cancer chemotherapeutic agents generate only modest amounts of ROS/RNS. In contrast to the anthracycline antibiotics, platinum-coordination complexes and camptothecins, the taxanes (e.g., paclitaxel and docetaxel), vinca alkaloids (e.g., vincristine and vinblastine), anti-metabolites, such as the antifolates, and nucleoside and nucleotide analogues generate only low levels of oxidative stress [36-38]. They do, however, generate some oxidative stress, as do all antineoplastic agents, when they induce apoptosis in cancer cells. This occurs when drug-

induced apoptosis is triggered by the release of cytochrome c from the mitochondrial electron transport chain. When this occurs, electrons are diverted from NADH dehydrogenase and reduced CoQ₁₀ to oxygen, resulting in the formation of superoxide radicals [39].

During cancer chemotherapy drug-induced oxidative stress produces side effects and reduces the anticancer efficacy of therapy [36]. Antineoplastic agents have clearly established mechanisms of action that do not depend upon the generation of ROS/RNS [38]. However, the drugs only exert their anticancer effects on cancer cells that exhibit unrestricted progression through their cell cycle and have intact apoptotic pathways. Oxidative stress interferes with cell cycle progression by inhibiting the transition of cells from the G₀ (quiescent) to the G₁ phase, slowing progression through the S phase by inhibition of DNA synthesis, inhibiting cell cycle progression through the restriction point (preventing G₁ phase to S phase transition), and by causing checkpoint arrest [40-46].

Thus the effects of oxidative stress diminish the cytotoxicity of anthracyclines and epipodophyllotoxins that act in the S phase and inhibit topoisomerase II activity as well as antifolates and nucleotide/nucleoside analogues that also act in the S phase and interfere with DNA synthesis. In contrast, vinca alkaloids and taxanes act primarily during the M phase and interfere with the mitotic process, whereas camptothecins act in the S phase and inhibit topoisomerase I activity. Platinum coordination complexes and alkylating agents, which are not considered to be phase-specific agents, still require cells to progress through the S phase and G₂ phase of the cell cycle in order for apoptosis to occur [44, 45].

DNA repair of damage caused by alkylating agents and platinum coordination complexes results in resistance to these drugs, and checkpoint arrest during oxidative stress can enhance the repair processes and diminish the efficacy of the treatment [47-49]. Interestingly, checkpoint abrogation--the opposite of what occurs during oxidative stress--enhances the cytotoxicity of antineoplastic agents. By reducing oxidative stress, antioxidants counteract the effects of chemotherapy-induced oxidative stress on the cell cycle and enhance the cytotoxicity of antineoplastic agents [36].

Oxidative stress also interferes with drug-induced apoptosis, important intracellular signal transduction pathways that are necessary for some antineoplastic agents [50] to exert their cytotoxic effect on cancer cells. The two major pathways of drug-induced apoptosis following cellular damage by antineoplastic agents are the mitochondrial pathway, initiated by release of

cytochrome c, and the CD95 death receptor pathway, initiated by binding to the death receptor of its ligand CD95L [48]. The proapoptotic signals of CD95 ligation or cytochrome c release activate initiator caspases that subsequently activate effector caspases that carry out disassembly of the cell. Oxidative stress during chemotherapy results in the generation of highly electrophilic aldehydes that have the ability to bind to the nucleophilic active sites of caspases as well as the nucleophilic extracellular domain of the CD95 death receptor. This inhibits caspase activity and the binding of CD95L ligand, thus interfering with the ability of antineoplastic agents to initiate apoptotic cell death [50-53].

Mitochondrial damage induced by anthracyclines

Cardiac mitochondria are especially sensitive to chemotherapy with anthracycline antibiotics [55]. Anthracycline-induced cardiac toxicity is characterized by acute, reversible toxicity that causes electrocardiographic changes and depressed myocardial contractility and by chronic, irreversible, dose-related cardiomyopathy [reviewed in 36]. The selective toxicity to cardiac cells that is caused by anthracyclines is due to disruption and damage of cardiac mitochondria. The unique sensitivity of cardiac cells to damage by anthracyclines is due a structural component of the electron transport system in cardiac mitochondria that is not present in mitochondria of other tissues and organs. Specifically, cardiac mitochondria are unique from mitochondria of other cell types in that they possess a Complex I-associated NADH dehydrogenase that faces the mitochondrial cytosol [56, 57].

Anthracyclines like doxorubicin possess a hexose sugar (daunosamine) attached to a tetracycline structure containing adjacent quinone and hydroquinone moieties that permit this class of drug to participate in oxidation-reduction reactions. Due to its small molecular weight (580 d) doxorubicin readily penetrates the outer mitochondrial membrane, but because of its hydrophilic properties it cannot penetrate the inner membrane. Thus, it cannot participate in oxidation-reduction reactions with the matrix-facing dehydrogenases of the electron transport chain found in most types of cells, such as liver, kidney and tumor cells [56-58]. In cardiac cells, however, doxorubicin interacts with the cytosolic-facing NADH dehydrogenase that is unique to cardiac mitochondria, resulting in reduction of the drug to its semiquinone [59-62]. The semiquinone is then auto-oxidized to the fully reduced dihydroquinone, and this reaction

destabilizes the molecule resulting in cleavage of the sugar moiety and formation of doxorubicin aglycones [62].

The aglycones of doxorubicin are highly lipid soluble and readily penetrate the inner mitochondrial membrane where they displace CoQ₁₀ from the electron transport chain. Thus when doxorubicin is administered *in vivo*, there is an increase in the plasma concentration of CoQ₁₀ [63] and a decrease in the content of CoQ₁₀ in cardiac muscle [64]. Once doxorubicin aglycones displace CoQ₁₀ from the mitochondrial inner membrane, they serve as electron acceptors from Complex I and Complex II. CoQ₁₀ normally accepts electrons from Complexes I and II and transfers them down the chain resulting in the formation of water. However, the aglycones transfer the electrons directly to molecular oxygen leading to the formation of superoxide radicals [62]. Therefore, doxorubicin generates an exceptionally high level of oxidative stress in cardiac mitochondria, interfering with cellular energetics (acute cardiac toxicity) and also resulting in severe damage to mitochondrial DNA [65, 66].

Anthracycline damage to mitochondrial DNA blocks the synthesis of mitochondrial ribosomal and transfer RNA that are necessary for the regenerative processes of the mitochondria, including the synthesis of electron transport chain components [67]. The inability of anthracycline-damaged mitochondria to sustain their structure and function results in disruption of cardiac cell mitochondria, resulting in cardiomyocyte apoptosis. Loss of these contractile cells of the heart causes cardiac insufficiency that does not respond to pharmacological interventions. Ultimately this may result in cardiac failure requiring the patient to undergo a heart transplantation. However, if CoQ₁₀ is administered during chemotherapy with anthracyclines, it prevents damage to the heart by decreasing anthracycline metabolism within cardiac mitochondria and by competing with anthracycline aglycones for the CoQ₁₀ site within the electron transport chain. Thus, it has been hypothesized that CoQ₁₀ administered concurrently with anthracyclines maintains the integrity of cardiac mitochondria and prevents damage to the heart while also enhancing the anti-cancer activity of the anthracyclines by diminishing their catabolism.

Molecular Replacement of CoQ₁₀ during anthracycline chemotherapy: preclinical data

Molecular Replacement of CoQ₁₀ dramatically prevents development of anthracycline-induced cardiomyopathy and histopathological changes in animal studies. For example, rabbits given IV

doxorubicin at a dose of 1 mg/kg 3 times weekly every other week for a total of 4 months develop severe histological changes in heart tissue that are characteristic of doxorubicin-induced cardiomyopathy. The rabbits also showed marked EKG changes and elevations in the level of creatine phosphokinase [68]. When 2.5 mg/kg CoQ₁₀ was administered IV with each dose of doxorubicin to another group of rabbits, the animals developed only very minimal histological changes in the heart and exhibited only minimal changes in their EKG patterns. The same protocol for doxorubicin and CoQ₁₀ administration was used in another study, except that CoQ₁₀ was not administered until a total of 15 mg/kg of doxorubicin had been given. Injections IV were then continued until a total of 30 mg/kg of doxorubicin was administered. The administration of CoQ₁₀ resulted in increased survival, improvement in the EKG patterns observed after the initial 15 mg/kg of doxorubicin, and reduced histopathological changes in the heart [69]. These findings indicate that CoQ₁₀ administration during chemotherapy can prevent the cardiomyopathic changes induced by doxorubicin.

Further evidence for a cardioprotective effect of CoQ₁₀ during doxorubicin therapy was seen in a longer study. Rabbits were given doxorubicin IV (0.8 mg/kg) on 3 consecutive days each week for 3 months. The treatment resulted in histopathological changes in the heart and EKG changes (flattened/inverted T waves and decreased QRS voltage) that are characteristic of doxorubicin-induced cardiomyopathy [74]. CoQ (at doses of 0.1 or 0.4 mg/kg) given IV 5 days a week beginning with the first doxorubicin injection significantly reduced the histopathological and EKG changes induced by the drug.

Using rats chronic administration of doxorubicin IP (2 mg/kg once weekly for 18 weeks) resulted in histological changes of the heart that are characteristic of doxorubicin-induced cardiomyopathy [71]. As in rabbits, the administration of CoQ₁₀ (10 mg/kg IM 6 days per week) to rats prevented the development of cardiomyopathic changes in the doxorubicin-treated animals [71].

The above preclinical data support the contention that CoQ₁₀ protects the heart from anthracycline-induced cardiotoxicity. However, the impact of CoQ₁₀ on the antineoplastic efficacy of anthracyclines has not been studied.

Molecular Replacement of CoQ₁₀ during anthracycline chemotherapy: clinical data

The concurrent administration of CoQ₁₀ during chemotherapy can affect both acute and chronic cardiotoxicity caused by anthracyclines. For example, the importance of administering CoQ₁₀ on the development of doxorubicin-induced cardiotoxicity in patients with lung cancer was investigated by Judy et al. [72]. Fourteen adult patients with normal resting cardiac function received 50-70 mg/m² IV of doxorubicin at regular intervals, or doxorubicin plus 100 mg/day of CoQ₁₀ PO, beginning 3-5 days before the first dose of doxorubicin and continuing until therapy was complete. After a total cumulative dose of 600 mg/m² doxorubicin, the patients receiving doxorubicin alone exhibited marked impairment of cardiac function with a significant increase in heart rate and a substantial decrease in ejection fraction, stroke index and cardiac index. However, in patients receiving 600 mg/m² of doxorubicin IV along with CoQ₁₀ PO, cardiac function remained unchanged from that measured before therapy was started. Additionally, the patients taking CoQ₁₀ continued to receive doxorubicin until they received a total of 900 mg/m², a dose at which approximately 50% of patients treated with doxorubicin alone can be expected to develop cardiomyopathy with congestive heart failure [55]. Following administration of 900 mg/m² in those patients taking CoQ, the only change in cardiac function was a modest increase in heart rate, whereas ejection fraction, stroke index and cardiac index were unchanged from that measured before therapy was started. This study demonstrated that CoQ₁₀ prevents doxorubicin-induced cardiomyopathy and that the total cumulative dose of doxorubicin can be escalated when CoQ₁₀ is administered concurrently with the chemotherapeutic drug.

Other studies confirm the results of Judy et al. [72]. For example, Cortes et al. [73, 77, 78] measured systolic time intervals (the pre-ejection period/left ventricular ejection time) in 18 adult patients treated with 50 mg/m² doxorubicin (total cumulative dose of 200-500 mg/m²) plus vincristine and cyclophosphamide every 4 weeks. Eight of the 10 patients receiving chemotherapy alone exhibited a progressive prolongation of their systolic time intervals, indicating depressed left ventricular cardiac function, with increasing cumulative doses of doxorubicin, while two patients developed congestive heart failure after 200 and 350 mg/m² of doxorubicin. Only 2 of 8 patients receiving chemotherapy plus 50 mg/day of oral CoQ₁₀ showed an increase in systolic time interval, although one patient developed heart failure after 350 mg/m² of doxorubicin. Although these investigators used only small doses of CoQ₁₀, the results indicated that CoQ₁₀ can reduce the cardiac toxicity of doxorubicin.

Cardiac protection has also been seen in children treated with anthracyclines plus oral CoQ₁₀. Iarussi et al. [79] measured cardiac function in children with hematological malignancies who were treated with equal amounts of doxorubicin and daunorubicin (mean cumulative combined dose: 240 mg/m²) or with anthracyclines (mean cumulative combined dose: 252 mg/m²) plus 100 mg of oral CoQ₁₀ twice daily for the duration of the study. Cardiac function was evaluated by echocardiographic evaluation before therapy started, after a cumulative anthracycline dose of 180 mg/m² and at the completion of therapy. They found that left ventricular function was reduced in both groups (10 children in each group), although it occurred later and to a lesser degree in patients receiving oral CoQ₁₀ [75].

Investigators have seen consistent differences in cardiac output between patients who received oral CoQ₁₀ during anthracycline therapy and those that did not. For example, Folkers et al. [75] measured cardiac output before and during treatment of 6 adults with lung cancer receiving doxorubicin every 3-4 weeks (3-5 infusions, total cumulative dose of 250-361 mg), or 4 patients receiving doxorubicin (total cumulative dose of 215-355 mg) plus 60 mg/day oral CoQ₁₀, or two infusions of doxorubicin (total cumulative dose of 145-175 mg) plus 60 mg/day oral CoQ₁₀. The patients who received doxorubicin without CoQ₁₀ showed a 25-40% reduction in cardiac output following the second or third drug infusion. However, in patients receiving CoQ₁₀, one exhibited a 16% reduction of cardiac output following the fourth doxorubicin infusion, one exhibited an 18% reduction of cardiac output following the third infusion, and one had a transient reduction of cardiac output following the second infusion that resolved. The remaining patients showed no change in cardiac output during treatment. Thus the majority of patients in these studies maintained their cardiac output when CoQ₁₀ was added during chemotherapy treatment [75].

In addition to cardiac output, changes in EKG profiles have been seen during anthracycline therapy that are prevented by oral CoQ₁₀. Okuma and Ota [76] randomized 80 cancer patients to receive doxorubicin (total cumulative dose 118-517 mg) or doxorubicin (total cumulative dose 123-517 mg) plus oral CoQ₁₀ (90 mg/day). Patients receiving doxorubicin alone had significant myocardial depression of the QRS voltage beginning with the first infusion and a significant prolongation of the Q-T interval after the fifth infusion. However, significant changes in the QRS voltage or the Q-T interval did not occur in patients receiving doxorubicin plus CoQ₁₀. Takimoto et al. [77] investigated the impact of oral CoQ₁₀ (90 mg/day) in a

randomized study of 40 cancer patients who were treated with doxorubicin (50 mg/m^2), cyclophosphamide, 5-fluorouracil plus radiation therapy. They found that administration of CoQ₁₀ reduced the frequency and severity of changes in the QRS complex, S-T segment, and T-wave, and the frequency of arrhythmias.

Although limited in number, the above clinical studies support the preclinical data that suggest that CoQ₁₀ protects the heart from the cardiotoxicity of anthracyclines. However, like preclinical studies, the impact of CoQ₁₀ on the antineoplastic efficacy of anthracycline-based chemotherapy has not been studied.

Cancer fatigue, aging and oxidative damage to mitochondria

Fatigue is usually the most common complaint of cancer patients undergoing therapy, but other complaints include pain, nausea, vomiting, malaise, diarrhea, headaches, rashes and infections. Other more serious problems can also occur, such as cardiomyopathy, peripheral neuropathy, hepatotoxicity, pulmonary fibrosis, mucositis and other effects [78, 79]. Interestingly, most patients felt that cancer therapy-associated fatigue was untreatable [80]. Although fatigue is often the most commonly reported adverse symptom during cancer therapy, there has been little effort directed at reducing fatigue [81]. Therefore, reducing fatigue associated with cancer therapy is an important goal, and nutritional methods have been undertaken to reduce fatigue and improve the quality of life of cancer patients [82]. Although fatigue in cancer patients has been defined as a multidimensional sensation [83], most patients understand fatigue as a loss of energy and inability to perform even simple tasks without exertion [83, 84].

At the tissue level fatigue is related to reductions in the efficiency of cellular energy systems in mitochondria [82, 85]. Damage to mitochondrial components, mainly by oxidation, can impair mitochondrial function, resulting in oxidative stress caused by over-production of ROS/RNS [reviews: 1, 5, 8]. Mitochondrial membranes and DNA are major targets of oxidative stress, and with aging ROS/RNS mitochondrial damage accumulates [86, 87].

In addition to aging, oxidative damage impairs mitochondrial function resulting in chronic fatigue. For example, in chronic fatigue syndrome (CFS) patients there is evidence of oxidative damage to DNA and lipids [88, 89] as well as oxidized blood markers [90] and muscle membrane lipids [91] that are indicative of excess oxidative stress [90]. CFS patients also have

sustained elevated levels of peroxynitrite due to excess nitric oxide, which can result in lipid peroxidation and loss of mitochondrial function as well as changes in cytokine levels that exert a positive feedback on nitric oxide production [92].

Molecular Replacement of oxidized membrane components

Membranes are especially sensitive to oxidative damage by ROS/RNS. Membrane phospholipid oxidation modifies their structure, affecting lipid fluidity, permeability and membrane function [93, 94]. One of the most important changes caused by ROS/RNS damage is loss of electron transport function, and this appears to be directly related to mitochondrial membrane lipid peroxidation, which induces permeability changes in mitochondria and loss of transmembrane potential, an essential requirement of mitochondrial oxidative phosphorylation [95, 96].

Lipid Replacement Therapy [82, 85] plus antioxidants has been used to reverse ROS/RNS damage and increase mitochondrial function in certain clinical disorders, such as chronic fatigue, CFS and Fibromyalgia Syndrome [82, 97]. Lipid Replacement Therapy has been found to be effective in preventing ROS/RNS-associated changes and reversing mitochondrial damage and loss of function [97, 98].

Molecular/Lipid Replacement Therapy: preclinical and clinical data

Oral Molecular/Lipid Replacement Therapy with unoxidized lipids and antioxidants has been effective in replacement of damaged cellular and mitochondrial membrane phospholipids and other lipids that are essential structural and functional components of all biological membranes [98, 99]. NTFactor®, a Lipid Replacement oral supplement containing phospholipids, phosphoglycolipids, cardiolipids and other membrane lipids, has been used successfully in animal and clinical lipid replacement studies [97-100]. NTFactor's encapsulated lipids are protected from oxidation in the gut and can be absorbed and transported into tissues without oxidation.

In preclinical studies NTFactor has been used to reduce age-related damage in rodents. Seidman et al. [100] found that NTFactor prevented hearing loss associated with aging and shifted the threshold hearing from 35-40 dB in control, aged animals to 13-17 dB. They also

found that NTFactor preserved cochlear mitochondrial function. NTFactor also prevented aging-related mitochondrial DNA deletions found in the cochlear [100]. Thus NTFactor was successful in preventing age-associated hearing loss and reducing mitochondrial damage in rodents.

In clinical studies Molecular/Lipid Replacement Therapy has been used to reduce fatigue and protect cellular and mitochondrial membranes from damage by ROS/RNS [97-99]. A vitamin supplement mixture containing NTFactor was by used by Ellithorpe et al. [99] in a dietary Molecular Replacement study of 34 patients with severe chronic fatigued patients to reduce their fatigue by approximately 40.5% in 8 weeks. In these studies fatigue was monitored by use of the Piper Fatigue Scale to measure clinical fatigue and quality of life [83]. In addition, in a subsequent study we examined the effects of NTFactor on fatigue and mitochondrial function in 20 patients [98]. Oral administration of NTFactor for 12 weeks resulted in a 35.5% reduction in fatigue [98]. In this clinical trial there was good correspondence between reductions in fatigue and gains in mitochondrial function, and after 12 weeks of supplementation, mitochondrial function was found to be similar to that of young healthy adults. In contrast, after a 12-week wash-out period fatigue increased and mitochondrial function decreased [98]. Thus in fatigued subjects dietary Molecular/Lipid Replacement Therapy can significantly improve and even restore mitochondrial function and significantly improve fatigue. Similar findings were observed in CFS and Fibromyalgia Syndrome patients [97].

Molecular/Lipid Replacement Therapy during cancer chemotherapy

Molecular/Lipid Molecular Replacement Therapy plus antioxidants has been used for reducing the adverse effects of chemotherapy in cancer patients. For example, Propax (a vitamin-mineral mixture with NTFactor) has been used in cancer patients to reduce some of most common adverse effects of cancer therapy, such as chemotherapy-induced fatigue, nausea, vomiting, malaise, diarrhea, headaches and other side effects [101]. In two studies conducted by Colodny *et al.* [101] on 38 advanced metastatic colon, pancreatic or rectal cancer patients receiving 5-FU/methotrexate/Leukovorin therapy on a 12-week schedule Molecular/Lipid Replacement was used to reduce adverse therapy effects. In the first unblinded part of the study the effectiveness of Propax with NTFactor administered before and during chemotherapy was determined by examining the signs/symptoms and side effects of therapy. A quality of life evaluation was

conducted by a research nurse, and it was determined that patients on NTFactor supplementation experienced significantly fewer episodes of fatigue, nausea, diarrhea, constipation, skin changes, insomnia and other effects. In contrast, no changes or a worsening were noted in the occurrence of sore throat or other indications of infection. In this open label trial 81% of patients demonstrated an overall improvement in quality of life parameters while on chemotherapy [101]. In the double-blinded, cross-over, placebo-controlled, randomized part of the study on advanced cancers the patients on Molecular/Lipid Molecular Replacement Therapy showed improvements in signs/symptoms associated with the adverse effects of chemotherapy [101]. Molecular/Lipid Molecular Replacement Therapy resulted in improvements in incidence of fatigue, nausea, diarrhea, impaired taste, constipation, insomnia and other quality of life indicators. Following cross-over from the placebo arm to the supplement arm, 57-70% of patients reported rapid improvements in nausea, impaired taste, tiredness, appetite, sick feeling and other quality of life indicators [101]. This clinical trial clearly demonstrated the usefulness of Molecular/Lipid Molecular Replacement Therapy and antioxidants given during chemotherapy.

Summary

Oral Molecular Replacement Therapy during cancer chemotherapy of metastatic disease can significantly reduce the adverse effects of cytotoxic drugs and limit the oxidative stress-related damage to normal cellular structures. Molecular Replacement supplements can be used to replace normal cellular constituents that are damaged as a therapeutic consequence of excess oxidative stress as well as those damaged due to aging and chronic disease. Molecular Replacement Therapy does not modify the anti-cancer cell therapeutic properties of chemotherapy drugs, but it does help protect normal cells and thus increases cancer therapeutic ratio. We conclude that Molecular Replacement Therapy is a cost-effective and safe method to reduce the adverse chronic and acute effects of cancer chemotherapy and improve clinical outcome [37].

References

1. Kehrer JP (1993) Free radicals and mediators of tissue injury and disease. *Crit Rev Toxicol* 23:21-48.

2. Halliwell B (1996) Oxidative stress, nutrition and health. *Free Rad Res* 25:57-74.
3. Dreher D, Junod AF (1996) Role of oxygen free radicals in cancer development. *Eur J Cancer* 32A:30-38.
4. Abidi S, Ali A (1999) Role of oxygen free radicals in the pathogenesis and etiology of cancer. *Cancer Lett* 142:1-9.
5. Stadtman E (2002) Introduction to serial reviews on oxidatively modified proteins in aging and disease. *Free Rad Biol Med* 32:789.
6. Marnett LJ (2000) Oxyradicals and DNA damage. *Carcinogenesis* 21:361-370.
7. Bartsch H, Nair J (2004) Oxidative stress and lipid peroxidation-driven DNA-lesions in inflammation driven carcinogenesis. *Cancer Detect Prevention* 28:385-391.
8. Castro L, Freeman BA (2001). Reactive oxygen species in human health and disease. *Nutrition* 17:295-307.
9. Johnson TM, Yu ZX, Ferrans VJ, et al. (1996) Reactive oxygen species are downstream mediators of p53-dependent apoptosis. *Proc Natl Acad Sci USA* 93:11848-11852.
10. Klaunig JE, Kamendulis LM (2004) The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicol* 44:239-267.
11. Barber DA, Harris SR (1994) Oxygen free radicals and antioxidants: a review. *Am Pharm* 34:26-35.
12. Sun Y (1990) Free radicals, antioxidant enzymes and carcinogenesis. *Free Rad Biol Med* 8:583-599.
13. Fridovich I (1995) Superoxide radical and superoxide dismutases. *Annu Rev Biochem* 64:97-112.
14. Seifried HE, McDonald SS, Anderson DE, et al. (2003) The antioxidant conundrum in cancer. *Cancer Res* 61:4295-4298.
15. Jagetia GC, Rajanikant GK, Rao SK, et al. (2003) Alteration in the glutathione, glutathione peroxidase, superoxide dismutase and lipid peroxidation by ascorbic acid in the skin of mice exposed to fractionated gamma radiation. *Clinica Chimica Acta* 332:111-121.
16. Schwartz JL (1996) The dual roles of nutrients as antioxidants and prooxidants: their effects on tumor cell growth. *J Nutr* 126:1221S-1227S.
17. Aeschbach R, Loliger J, Scott BC, et al. (1994) Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food Chem Toxicol* 32:31-36.

18. Tanaka T (1994) Cancer chemoprevention by natural products. *Oncol Rep* 1:1139-1155.
19. Prasad KN, Cole WC, Kumar B, et al. (2001) Scientific rationale for using high-dose multiple micronutrients as an adjunct to standard and experimental cancer therapies. *J Amer Coll Nutrition* 20:450S-453S.
20. Toyokuni S, Okamoto K, Yodio J, et al. (1995) Persistent oxidative stress in cancer. *FEBS Lett* 358:1-3.
21. Klaunig JE, Kamendulis LM (2004) The role of oxidative stress in carcinogenesis. *Annu Rev Pharmacol Toxicol* 44:239-267.
22. Brown NS, Bicknell R (2001). Hypoxia and oxidative stress in breast cancer. Oxidative stress: its effects on the growth, metastatic potential and response to therapy of breast cancer. *Breast Cancer Res* 3:323-327.
24. Ray G, Batra S, Shukla NK, et al. (2000). Lipid peroxidation, free radical production and antioxidant status in breast cancer. *Breast Cancer Res Treat* 59:163-170.
25. Tas F, Hansel H, Belce A, et al. (2005) Oxidative stress in breast cancer. *Med Oncol* 22:11-15.
26. Asal NR, Risser DR, Kadamani S, et al. (1990) Risk factors in renal cell carcinoma. I. Methodology, demographics, tobacco beverage use and obesity. *Cancer Detect Prev* 11:359-377.
27. Gago-Dominguez M, Castelao JE, Yuan JM, et al. (2002) Lipid peroxidation: a novel and unifying concept of the etiology of renal cell carcinoma. *Cancer Causes Control* 13:287-293.
28. Sikka SC (2003) Role of oxidative stress response elements and antioxidants in prostate cancer pathobiology and chemoprevention—a mechanistic approach. *Curr Med Chem* 10:2679-2692.
29. Aydin A, Arsova-Saradinovska Z, Sayal A, et al. (2006) Oxidative stress and antioxidant status in non-metastatic prostate cancer and benign prostate hyperplasia. *Clin Biochem* 39:176-179.
30. Otamiri T, Sjudahl R (1989) Increased lipid peroxidation in malignant tissues of patients with colorectal cancer. *Cancer* 64:422-425.
31. Oxdemirler G, Pabuccoglu H, Bulut T, et al. (1989) Increased lipoperoxide levels and antioxidant system in colorectal cancer. *J Cancer Res Clin Oncol* 124:555-559.

32. Manoharan S, Kolanjiappan K, Suresh K, et al. (2005) Lipid peroxidation and antioxidants status in patients with oral squamous cell carcinoma. *Ind J Med Res* 2005; 122: 529-534.
33. Seril DN, Liao J, Yang GY, et al. (2003) Oxidative stress and ulcerative colitis-associated carcinogenesis: studies in humans and animal models. *Carcinogenesis* 34:353-362.
34. Batcioglu K, Mehmet N, Ozturk IC, et al. (2006) Lipid peroxidation and antioxidant status in stomach cancer. *Cancer Investig* 24:18-21.
35. Jaruga P, Zastawny TH, Skokowski J, et al. (1992) Oxidative DNA base damage and antioxidant enzyme activities in human lung cancer. *FEBS Lett* 341:59-64.
36. Conklin KA (2004) Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. *Intgr Cancer Ther* 3:294-300.
37. Nicolson GL, Conklin KA (2006) Molecular replacement for cancer metabolic and mitochondrial dysfunction, fatigue and the adverse effects of cancer therapy. *Cancer Genomics Proteomics* 3:159-168.
38. Conklin KA (2000) Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects. *Nutr Cancer* 37:1-18.
39. Betteridge DJ (2000) What is oxidative stress? *Metabolism* 49(suppl 1):3-8.
40. Esterbauer H, Schaur RJ, Zollner H (1991) Chemistry and biochemistry of 4-hydroxynonenals, malonaldehyde and related aldehydes. *Free Radic Biol Med* 11:81-128.
41. Dianzani MU (1993) Lipid peroxidation and cancer. *Crit Rev Oncol Hematol* 15:125-147.
42. Hauptlorenz S, Esterbauer H, Moll W, et al. (1985) Effects of the lipid peroxidation product 4-hydroxynonenal and related aldehydes on proliferation and viability of cultured Ehrlich ascites tumor cells. *Biochem Pharmacol* 34:3803-3809.
43. Gonzalez MJ (1992) Lipid peroxidation and tumor growth: an inverse relationship. *Med Hypotheses* 38:106-110.
44. Schackelford RE, Kaufmann WK, Paules RS (2000) Oxidative stress and cell cycle checkpoint function. *Free Rad Biol Med* 28:1387-1404.
45. Balin AK, Goodman DBP, Rasmussen H, et al. (1978) Oxygen-sensitive stages of the cell cycle of human diploid cells. *J Cell Biol* 78:390-400.
46. Kurata S (2000) Selective activation of p38 MAPK cascade and mitotic arrest caused by low level oxidative stress. *J Biol Chem* 275:23413-23416.

47. Wei Q, Frazier ML, Levin B (2000) DNA repair: a double edge sword. *J Natl Can Inst* 92:440-441.
48. Fojo T (2001) Cancer, DNA repair mechanisms, and resistance to chemotherapy. *J Natl Can Inst* 93:1434-1436.
49. Zhen W, Link CJ, O'Connor PM, et al. (1992) Increased gene-specific repair of cisplatin interstrand cross-links in cisplatin-resistant human ovarian cancer cell lines. *Molec Cell Biol* 12:3689-3698.
50. Lee Y-J, Shacter E (1999) Oxidative stress inhibits apoptosis in human lymphoma cells. *J Biol Chem* 274:19792-19798.
51. Hampton MB, Orrenius S (1997) Dual regulation of caspase activity by hydrogen peroxide: implications for apoptosis. *FEBS Lett* 414:552-556.
52. Hampton MB, Fadeel B, Orrenius S (1998) Redox regulation of the caspases during apoptosis. *Ann New York Acad Sci* 854:328-335.
53. Chandra J, Samali A, Orrenius S (2000) Triggering and modulation of apoptosis by oxidative stress. *Free Rad Biol Med* 29:323-333.
54. Shacter E, Williams JA, Hinson RM, et al. (2000) Oxidative stress interferes with cancer chemotherapy: inhibition of lymphoma cell apoptosis and phagocytosis. *Blood* 96:307-313.
55. Conklin KA (2005) Coenzyme Q₁₀ for prevention of anthracycline-induced cardiotoxicity. *Intgr Cancer Ther* 4:110-130.
56. Lehninger AL (1951) Phosphorylation coupled to oxidation of dihydridiphosphopyridine nucleotide. *J Biol Chem* 190:345-359.
57. Rasmussen UF, Rasmussen HN (1985) The NADH oxidase system (external) of muscle mitochondria and its role in the oxidation of cytoplasmic NADH. *Biochem J* 229:632-641.
58. Nohl H (1987) Demonstration of the existence of an organo-specific NADH dehydrogenase in heart mitochondria. *Eur J Biochem* 169:585-591.
59. Davies KJA, Doroshov JH (1986) Redox cycling of anthracyclines by cardiac mitochondria. I. Anthracycline radical formation by NADH dehydrogenase. *J Biol Chem* 261:3060-3067.
60. Doroshov JH, Davies KJA (1986) Redox cycling of anthracyclines by cardiac mitochondria. II. Formation of superoxide anion, hydrogen peroxide, and hydroxyl radical. *J Biol Chem* 261:3068-3074.

61. Nohl H (1988) Identification of the site of Adriamycin-activation in the heart cell. *Biochem Pharmacol* 37:2633-2637.
62. Gille L, Nohl H (1997) Analyses of the molecular mechanism of Adriamycin-induced cardiotoxicity. *Free Rad Biol Med* 23:775-782.
63. Eaton S, Skinner R, Hale JP, et al. (2000) Plasma coenzyme Q₁₀ in children and adolescents undergoing doxorubicin therapy. *Clin Chim Acta* 302:1-9.
64. Karlsson J, Folkers K, Astrom H, et al. (1986) Effect of Adriamycin on heart and skeletal muscle coenzyme Q₁₀ (CoQ₁₀) in man. In Folkers K, Yamamura Y (eds): *Biomedical and Clinical Aspects of Coenzyme Q*, vol 5, Amsterdam:Elsevier/North-Holland Biomedical Press, 241-245.
65. Palmeira CM, Serrano J, Kuehl DW, et al. (1997) Preferential oxidation of cardiac mitochondrial DNA following acute intoxication with doxorubicin. *Biochim Biophys Acta* 1321:101-106.
66. Serrano J, Palmeira CM, Kuehl DW, et al. (1999) Cardioselective and cumulative oxidation of mitochondrial DNA following subchronic doxorubicin administration. *Biochim Biophys Acta* 1411:201-205.
67. Papadopoulou LC, Tsiftoglou AS (1996) Effects of hemin on apoptosis, suppression of cytochrome C oxidase gene expression, and bone-marrow toxicity induced by doxorubicin. *Biochem Pharmacol* 52:713-722.
68. Domae N, Sawada H, Matsuyama E, et al. (1981) Cardiomyopathy and other chronic toxic effects induced in rabbits by doxorubicin and possible prevention by coenzyme Q₁₀. *Cancer Treat Rep* 65:79-91.
69. Usui T, Ishikura H, Izumi Y, et al. (1982) Possible prevention from the progression of cardiotoxicity in Adriamycin-treated rabbits by coenzyme Q₁₀. *Toxicol Lett* 12:75-82.
70. Ghione M, Bertazzoli C (1977) CoQ and anthracycline associated cardiomyopathy. In Folkers K, Yamamura Y (eds): *Biomedical and Clinical Aspects of Coenzyme Q*, Amsterdam: Elsevier/North-Holland Biomedical Press, 183-199.
71. Yamanaka N, Kato T, Nishida K, et al. (1980) Protective effect of coenzyme Q₁₀ on Adriamycin toxicity and increase of antitumor effects of Adriamycin by coenzyme Q₁₀. In Yamamura Y, Folkers K, Ito Y (eds): *Biomedical and Clinical Aspects of Coenzyme Q*, vol 2, Amsterdam:Elsevier/North-Holland Biomedical Press, 213-224.

72. Judy WV, Hall JH, Dugan W, et al. (1984) Coenzyme Q₁₀ reduction of Adriamycin cardiotoxicity. In Folkers K, Yamamura Y (eds): Biomedical and Clinical Aspects of Coenzyme Q, vol 4, Amsterdam:Elsevier/North-Holland Biomedical Press, 231-241.
73. Cortes EP, Gupta M, Chou C, et al. (1978) Adriamycin cardiotoxicity: early detection by systolic time interval and possible prevention by coenzyme Q₁₀. *Cancer Treat Rep* 62:887-891.
74. Iarussi, D, Auricchio, U, Agretto, A, et al. (1994) Protective effect of coenzyme Q₁₀ on anthracyclines cardiotoxicity: control study in children with acute lymphoblastic leukemia and non-Hodgkin lymphoma. *Molec Aspects Med* 15:S207-S212.
75. Folkers K, Baker L, Richardson PC, et al. (1981) New progress on the biomedical and clinical research on coenzyme Q₁₀. In Folkers K, Yamamura Y (eds): Biomedical and Clinical Aspects of Coenzyme Q, vol 3, Amsterdam:Elsevier/North-Holland Biomedical Press, 399-412.
76. Okuma K, Ota K (1986) The effect of coenzyme Q₁₀ on ECG changes induced by doxorubicin (Adriamycin). In Folkers K, Yamamura Y (eds): Biomedical and Clinical Aspects of Coenzyme Q, vol 5, Amsterdam:Elsevier/North-Holland Biomedical Press, 247-256.
77. Takimoto M, Sakurai T, Kodama K, et al. Protective effect of CoQ 10 administration on cardiac toxicity in FAC therapy. *Gan To Kagaku Ryoho* 1982; 9: 116-121.
78. Buckingham R, Fitt J, Sitzia J (1997) Patients' experience of chemotherapy: side-effects of carboplatin in the treatment of carcinoma of the ovary. *Eur J Cancer Care* 6:59-71.
79. Loke YK, Price D, Derry S, et al. (2006) Case reports of suspected adverse drug reactions—systematic literature survey of follow-up. *Br Med J* 232:335-339.
80. Vogelzang N, Breitbart W, Cella D, et al. (1997) Patient caregiver and oncologist perceptions of cancer-related fatigue: results of a tripart assessment survey. *Semin Hematol* 34(Suppl 2):4-12.
81. Von Roenn JH, Paice JA (2005) Control of common, non-pain cancer symptoms. *Semin Oncol* 32:200-210.
82. Nicolson GL (2005) Lipid replacement/antioxidant therapy as an adjunct supplement to reduce the adverse effects of cancer therapy and restore mitochondrial function. *Pathol Oncol Res* 11:139-144.

83. Piper BF, Linsey AM, Dodd MJ (1987) Fatigue mechanism in cancer. *Oncol Nursing Forum* 14:17-23.
84. McDonald E, David AS, Pelosi AJ, et al. (1993) Chronic fatigue in primary care attendees. *Psychol Med* 23:987-998.
85. Nicolson GL (2003) Lipid replacement as an adjunct to therapy for chronic fatigue, anti-aging and restoration of mitochondrial function. *J Am Nutraceut Assoc* 6(3):22-28.
86. Wei YH, Lee HC (2002) Oxidative stress, mitochondrial DNA mutation and impairment of antioxidant enzymes in aging. *Exp Biol Med* 227:671-682.
87. Huang H, Manton KG (2004) The role of oxidative damage in mitochondria during aging: a review. *Front Biosci* 9:1100-1117.
88. Logan AC, Wong C (2001) Chronic fatigue syndrome: oxidative stress and dietary modifications. *Altern Med Rev* 6:450-459.
89. Manuel y Keenoy B, Moorkens G, et al. (2001) Antioxidant status and lipoprotein peroxidation in chronic fatigue syndrome. *Life Sci* 68:2037-2049.
90. Richards RS, Roberts TK, McGregor NR, et al. (2000) Blood parameters indicative of oxidative stress are associated with symptom expression in chronic fatigue syndrome. *Redox Rep* 5:35-41.
91. Felle S, Mecocci P, Fano G, et al. (2000) Specific oxidative alterations in vastus lateralis muscle of patients with the diagnosis of chronic fatigue syndrome. *Free Radical Biol Med* 29:1252-1259.
92. Pall ML. Elevated, sustained peroxynitrite levels as the cause of chronic fatigue syndrome. *Med Hypotheses* 2000; 54: 115-125.
93. Nicolson GL, Poste G, Ji T (1977) Dynamic aspects of cell membrane organization. *Cell Surface Rev* 3:1-73.
94. Subczynski WK, Wisniewska A (2000) Physical properties of lipid bilayer membranes: relevance to membrane biological functions. *Acta Biochim Pol* 47:613-625.
95. Radi R, Rodriguez M, Castro L, et al. (1994) Inhibition of mitochondrial electronic transport by peroxynitrite. *Arch Biochem Biophys* 308:89-95.
96. Kanno T, Sato EE, Muranaka S, et al. (2004) Oxidative stress underlies the mechanism for Ca(2+)-induced permeability transition of mitochondria. *Free Radical Res* 38:27-35.

97. Nicolson GL, Ellithorpe R (2006) Lipid replacement and antioxidant nutritional therapy for restoring mitochondrial function and reducing fatigue in chronic fatigue syndrome and other fatiguing illnesses. *J Chronic Fatigue Syndr* 13(1):57-68.
98. Agadjanyan M, Vasilevko V, Ghochikyan A, et al. (2003) Nutritional supplement (NTFactor) restores mitochondrial function and reduces moderately severe fatigue in aged subjects. *J Chronic Fatigue Syndr* 11(3):23-26.
99. Ellithorpe RR, Settineri R, Nicolson GL (2003) Reduction of fatigue by use of a dietary supplement containing glycerophospholipids. *J Am Nutraceut Assoc* 6(1):23-28.
100. Seidman M, Khan MJ, Tang WX, et al. (2002) Influence of lecithin on mitochondrial DNA and age-related hearing loss. *Otolaryngol Head Neck Surg* 127:138-144.
101. Colodny L, Lynch K, Farber C, et al. (2000) Results of a study to evaluate the use of Propax to reduce adverse effects of chemotherapy. *J Am Nutraceut Assoc* 2(1):17-25.