

Molecular Replacement in Cancer Therapy: Reversing Cancer Metabolic and Mitochondrial Dysfunction, Fatigue and the Adverse Effects of Cancer Therapy

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Abstract:

Introduction: Cancers are associated with excess cellular oxidative stress, and during cancer treatment the addition of drug-induced oxidative stress can limit the effectiveness of therapy and cause a number of side effects, such as fatigue, nausea, vomiting and diarrhea, as well as more serious adverse effects, including cardiomyopathy, peripheral neuropathy, hepatotoxicity and pulmonary fibrosis.

Method: Review of the pertinent literature on oxidative stress during cancer cytotoxic therapy and the use of Molecular Replacement methods to reduce adverse effects by replacement of damaged cellular molecules.

Discussion: Most of the adverse effects of cancer therapy are due to oxidative stress-mediated damage to normal tissues. For example, loss of efficiency in the electron transport chain caused by membrane peroxidation and reduction in coenzyme Q₁₀ can occur during cytotoxic therapy using anthracyclines, alkylating agents, platinum coordination complexes, epipodophyllotoxins and camptothecins. Molecular Replacement and antioxidant administration mitigates the damage to normal tissues and reduces the adverse effects of cancer therapy without loss of therapeutic effect.

Summary: The acute and chronic adverse effects of cancer chemotherapy can be reduced by Molecular Replacement. Molecular Replacement of membrane lipids and enzymatic cofactors, such as coenzyme Q₁₀, by administering nutritional supplements with antioxidants can prevent oxidative membrane damage and reductions of cofactors in normal tissues, respectively, restoring mitochondrial and other cellular functions and reducing chemotherapy adverse effects, such as cardiotoxicity, without significantly affecting therapeutic benefit. Recent clinical trials using cancer and non-cancer patients with chronic fatigue have shown the benefit of Molecular Replacement Therapy 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.

Keywords: Oxidative stress, alkylating agents, anthracyclines, mitochondria, coenzyme Q10, lipid peroxidation, electron transport chain, antioxidants, lipid replacement.

INTRODUCTION

Excess cellular oxidative stress [1] is associated with the etiology of cancer as well as aging and age-related degenerative diseases [2-6]. Oxidative stress is caused by an excess of reactive oxygen (ROS) and nitrogen (NOS) species over cellular antioxidants, resulting in oxidation of cellular structures, such as membrane lipids and proteins [7, 8] and mutation of DNA [9-11]. ROS and NOS are naturally occurring cellular oxidants that are involved in cell proliferation, gene expression, intracellular signaling, antimicrobial defense and other normal cellular processes [12-14], and it is only when ROS/NOS are in excess that cellular damage occurs.

Cellular antioxidant defenses normally maintain ROS and NOS at appropriate physiological concentrations [15-17]. Endogenous cellular antioxidant defenses include the enzymes glutathione peroxidase, catalase, superoxide dismutase, among others [18, 19], and low molecular weight die-

tary antioxidants [20, 21]. These nutritional antioxidants have been used as natural chemopreventive agents [22, 23] to shift the balance of oxidative molecules towards more physiological levels.

The promotion and progression of cancer are linked to excess oxidative stress in many malignancies [24-26]. For example, oxidative stress and antioxidant status have been examined in various cancers, such as breast [25-29], renal [30, 31], prostate [32, 33], colorectal [34, 35] and other cancers [36-38]. In these studies the oxidative species were in excess of antioxidant properties of the cells, and these cancers were proposed to arise, in part, as a consequence of this imbalance and oxidative changes in the genetic apparatus [5, 6, 9-11, 39, 40].

CHEMOTHERAPY-INDUCED OXIDATIVE STRESS

Antineoplastic agents generate ROS in biological systems [41] (Table 1). 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

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Table 1. Antineoplastic Agents: Generation of Oxidative Stress

<u>Very high levels</u>
Anthracyclines
Doxorubicin (Adriamycin [®])
Daunorubicin
Epirubicin
<u>High levels</u>
Alkylating agents
Cyclophosphamide (Cytosan [®])
Ifosfamide
Platinum complexes
Cisplatin
Carboplatin
Oxaliplatin
Epipodophyllotoxins
Etoposide
Teniposide
Camptothecins
Topotecan
Irinotecan
<u>Low levels</u>
Taxanes
Paclitaxel (Taxol [®])
Docetaxel (Taxotere [®])
Vinca alkaloids
Vincristine (Oncovin [®])
Vinblastine (Velban [®])
Antifolates
Methotrexate
Nucleotide & nucleoside analogues
5-fluorouracil
Capecitabine (Xeloda [®])
Gemcitabine (Gemzar [®])

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 (Table 1). The cytochrome P450 monooxygenase system of hepatic microsomes is a primary site of ROS generation. 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 (CoQ) 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.

In contrast to the above groups of antineoplastic agents (Table 1), 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. Although they do not generate ROS at multiple sites within the cell, they do generate some oxidative stress, as do all antineoplas-

tic 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 CoQ to oxygen with formation of superoxide radicals.

Drug-induced oxidative stress during cancer chemotherapy produces side effects and reduces the anticancer efficacy of therapy [41]. Antineoplastic agents have clearly established mechanisms of action that do not depend upon the generation of ROS [42]. 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 [43-49] (Table 2). These effects of oxidative stress diminish the cytotoxicity of anthracyclines and epipodophyllotoxins that inhibit topoisomerase II activity and act in the S phase, antifolates and nucleotide/nucleoside analogues that interfere with DNA synthesis and act in the S phase, vinca alkaloids and taxanes that interfere with the mitotic process and act primarily during the M phase, and camptothecins that inhibit topoisomerase I activity and act in the S phase. 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. Additionally, DNA repair of damage caused by platinum coordination complexes and alkylating agents results in resistance to these drugs, and checkpoint arrest during oxidative stress can enhance the repair processes and diminish the efficacy of the treatment [50-52]. In this regard, 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.

Table 2. Oxidative Stress: Impact on Cell Cycle and Drug-Induced Apoptosis

<u>Cell Cycle Effects [43-49]</u>
Inhibits G ₀ to G ₁ transition
Inhibits DNA synthesis
Blocks restriction point transition
Causes checkpoint arrest
<u>Effects on Apoptosis [53-57]</u>
Inhibits caspase activity
Binds CD95 death receptor

In addition to the effects on cell cycle progression, oxidative stress also interferes with drug-induced apoptosis. 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 ligation of the death receptor by its ligand CD95L [51]. The proapoptotic

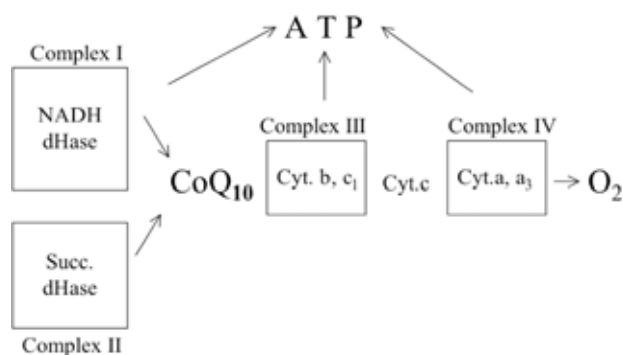


Fig. (1). The electron transport system. Electrons flow from Complex I (NADH dehydrogenase) and Complex II (succinate dehydrogenase) to coenzyme Q_{10} , then to Complex III (cytochrome b and c_1), cytochrome c, and Complex IV (cytochrome a and a_3). The final transfer is to molecular oxygen with the formation of water. The electron transport system is coupled at three sites to oxidative phosphorylation that results in the formation of adenosine triphosphate (ATP). From Ref. [41] with permission.

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 and the nucleophilic extracellular domain of the CD95 death receptor. This inhibits caspase activity and binding of CD96L, thereby interfering with the ability of antineoplastic agents to kill cancer cells by apoptotic mechanisms [53-57].

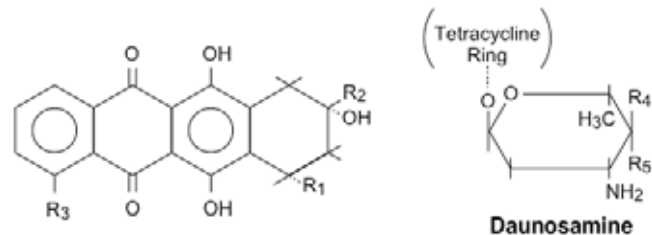
ANTHRACYCLINE-INDUCED MITOCHONDRIAL DAMAGE TO CARDIAC CELLS

Anthracycline antibiotics are associated with cardiac toxicity that is manifest by acute reversible toxicity (electrocardiographic changes and depressed myocardial contractility) and a chronic irreversible cardiomyopathy that is dose-related. The cellular damage by anthracyclines is selective for cardiac cells and is due to damage and disruption 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 [58].

The electron transport system in mitochondria of all cells (Fig. 1) is located within the inner mitochondrial membrane. Electron transport is initiated when reducing equivalents enter the system from Complex I (NADH dehydrogenase) and Complex II (succinate dehydrogenase). The enzymatic components of these complexes face the mitochondrial matrix that is enclosed within the inner mitochondrial membrane. Electron transfer continues from Complex I and Complex II to CoQ, Complex III, cytochrome c, and Complex IV. The final transfer is to molecular oxygen with the formation of water. The inner membrane separates the matrix from the mitochondrial cytosol. The cytosol is contained within the mitochondrial outer membrane. The outer membrane is permeable to molecules with a molecular weight of less than 10,000 d. The inner membrane is permeable only to small lipid soluble molecules and substances transferred by trans-

port mechanisms. Cardiac mitochondria are unique from mitochondria of other types of cells in that they possess a Complex I-associated NADH dehydrogenase that faces the mitochondrial cytosol [59, 60].

Doxorubicin, as other anthracyclines, possesses a hexose sugar (daunosamine) attached to a tetracycline structure containing adjacent quinone and hydroquinone moieties (Fig. 2) that permits the drug to participate in oxidation-reduction reactions. Although doxorubicin readily penetrates the outer mitochondrial membrane due to its small size (580 d), 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 in most types of cells including those of liver, kidney, and tumors [59-61]. In cardiac mitochondria, however, doxorubicin interacts with the cytosolic-facing NADH dehydrogenase that is unique to these mitochondria, resulting in reduction of the drug to its semiquinone [62-65]. Autooxidation results in formation of the fully reduced dihydroquinone, and this reaction destabilizes the molecule resulting in cleavage of the sugar moiety and formation of doxorubicin aglycones (Fig. 2) [65]. Aglycones of doxorubicin are highly lipid soluble and readily penetrate the inner membrane where they displace CoQ from the electron transport chain (Fig. 3). This is evident from the rise in the plasma CoQ level when doxorubicin is administered during chemotherapy [66] and by the marked decrease of the CoQ content of cardiac muscle following chemotherapy with doxorubicin [67].



ANTHRACYCLINES				
	Doxorubicin	Daunorubicin	Epirubicin	Idarubicin
R ₁	Daunosamine	Daunosamine	Daunosamine	Daunosamine
R ₂	$\text{-C(=O)-CH}_2\text{OH}$	-C(=O)-CH_3	$\text{-C(=O)-CH}_2\text{OH}$	-C(=O)-CH_3
R ₃	-OCH_3	-OCH_3	-OCH_3	-H
R ₄	-H	-H	-OH	-H
R ₅	-OH	-OH	-H	-OH
DOXORUBICIN METABOLITES				
	Doxorubicinol	Aglycone	Deoxyaglycone	
R ₁	Daunosamine	-OH	-H	
R ₂	$\text{-CH(OH)CH}_2\text{OH}$	$\text{-C(=O)-CH}_2\text{OH}$	$\text{-C(=O)-CH}_2\text{OH}$	
R ₃	-OCH_3	-OCH_3	-OCH_3	
R ₄	-H	_____	_____	
R ₅	-OH	_____	_____	

Fig. (2). The structures of anthracyclines and doxorubicin metabolites. From Ref. [58] with permission.

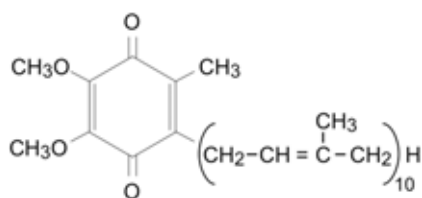


Fig. (3). The structure of coenzyme Q₁₀. From Ref. [58] with permission.

Once doxorubicin aglycones displace CoQ from the mitochondrial inner membrane, they serve as electron acceptors from Complex I and Complex II. However, whereas CoQ normally accepts electrons from these Complexes and transfers them down the chain resulting in the formation of water, the aglycones transfer electrons directly to molecular oxygen with the formation of superoxide radicals (Fig. 4) [65]. Thus, doxorubicin generates an exceptionally high level of oxidative stress in cardiac mitochondria. Acutely, this interferes with cellular energetics (acute cardiac toxicity), but it also results in severe damage to mitochondrial DNA [68, 69].

Damage to the mitochondrial genome by anthracyclines blocks synthesis of mitochondrial ribosomal and transfer RNA that is necessary for the regenerative processes of the organelle, including synthesis of electron transport chain components [70]. The inability of anthracycline-damaged mitochondria to sustain their structure and function leads to disruption of the mitochondria of cardiac cells and results in myocyte apoptosis. Loss of these contractile cells of the heart results in cardiac insufficiency that does not respond to pharmacological interventions, and this may result in cardiac failure requiring the patient to undergo a heart transplantation. Fortunately, CoQ administered during therapy with anthracyclines prevents damage to the heart by decreasing anthracycline metabolism within cardiac mitochondria and by competing with anthracycline aglycones for the CoQ site within the electron transport chain. Thus, CoQ administered concurrently with anthracyclines maintains the integrity of mitochondria and prevents damage to the heart while also enhancing the anti-cancer activity of the anthracyclines by diminishing their catabolism.

MOLECULAR REPLACEMENT OF COQ DURING ANTHRACYCLINE THERAPY: PRECLINICAL STUDIES

Molecular Replacement of CoQ dramatically prevents development of anthracycline-induced cardiomyopathy in animal studies. For example, rabbits given IV doxorubicin, 1 mg/kg 3 times weekly every other week for 4 months (maximum dose: 25 mg/kg) develop severe histological changes in the heart that are characteristic of doxorubicin-induced cardiomyopathy [71, 72]. The rabbits also exhibit marked EKG changes and elevation of the creatine phosphokinase level. When CoQ, 2.5 mg/kg IV, was administered 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 EKG changes. In another study [73], the same protocol for doxorubicin and CoQ administration was used except that CoQ was not administered until a total of 15 mg/kg of doxorubicin had been given. Injections were then continued until a total of 30 mg/kg of doxorubicin was administered. CoQ administration resulted in improved survival, improvement of the EKG changes observed after the initial 15 mg/kg of doxorubicin, and less histopathological changes in the heart. These findings suggest that CoQ can prevent the progression of cardiomyopathic changes induced by doxorubicin.

In another longer study rabbits given doxorubicin (0.8 mg/kg IV) on 3 consecutive days each week for 3 months, also 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 IV) given 5 days a week beginning with the first doxorubicin injection, significantly reduced the histopathological and EKG changes induced by the drug. These results provide further evidence that CoQ is cardioprotective during extended therapy with doxorubicin.

Chronic administration of IP doxorubicin (2 mg/kg once weekly for 18 weeks) to rats results in histological changes of the heart that are characteristic of doxorubicin-induced

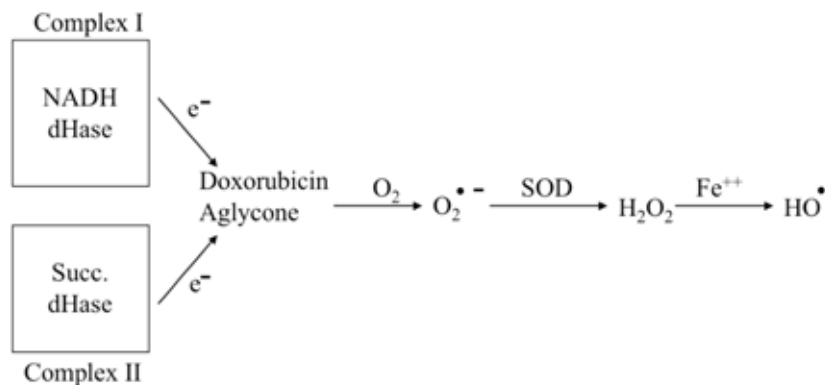


Fig. (4). The effect of doxorubicin aglycones on the electron transport system. Doxorubicin aglycones diffuse into the inner mitochondrial membrane and displace coenzyme Q₁₀. The aglycones accept electrons from Complex I (NADH dehydrogenase) and Complex II (succinate dehydrogenase) and transfer the electrons directly to molecular oxygen (instead of to Complex III), resulting in the formation of superoxide radicals (O₂^{•-}). Mitochondrial superoxide dismutase (SOD) then generates hydrogen peroxide and, in the presence of reduced iron (Fe⁺⁺) that is plentiful in mitochondria, high levels of hydroxyl radicals are formed.

Table 3. Coenzyme Q₁₀ and Doxorubicin Cardiotoxicity: Clinical Studies**Chronic Cardiotoxicity**

Reference	Doxorubicin ^a	Coenzyme Q ₁₀	Effect of Coenzyme Q ₁₀ ^b
Judy <i>et al.</i> [76]	600-900 mg/m ²	100 mg/day PO	Prevented decrease: EF, SI, CI/increase:HR Allowed escalation of doxorubicin dose
Cortes <i>et al.</i> [77, 78]	200-500 mg/m ²	50 mg/day PO	Reduced degree of STI prolongation
Iarussi <i>et al.</i> [79]	Mean: 240/252 mg/m ²	50 mg/day PO	Reduced degree of STI prolongation
Folkers <i>et al.</i> [80,81]	145-361 mg/m ²	60 mg/day PO	Reduced depression of CO
Okuma and Ota [82]	118-517 mg/m ²	90 mg/day PO	Prevented depression of QRS voltage/ Prevented prolongation of Q-T interval

^a Total cumulative dose^b Abbreviations: EF: ejection fraction; SI: stroke index; CI: cardiac index; HR: heart rate; STI: systolic time interval; LV: left ventricle; CO: cardiac output**Acute Cardiotoxicity**

Reference	Doxorubicin ^c	Coenzyme Q ₁₀	Effect of Coenzyme Q ₁₀ ^d
Takimoto <i>et al.</i> [83]	50 mg/m ²	90 mg/day PO	Prevented conduction abnormalities on EKG
Tsubaki <i>et al.</i> [84]	not stated	1 mg/kg/day IV	Prevented conduction abnormalities on EKG
Yamamura [85]	not stated	30 mg/day PO	Prevented conduction abnormalities on EKG

cardiomyopathy [75]. As in rabbits, administering CoQ (10 mg/kg IM 6 days per week) prevented the development of cardiomyopathic changes in the doxorubicin-treated rats.

MOLECULAR REPLACEMENT OF COQ DURING ANTHRACYCLINE THERAPY: CLINICAL STUDIES

CoQ can affect both acute and chronic cardiotoxicity caused by anthracyclines (Table 3). Judy *et al.* [76] investigated the impact of CoQ on the development of doxorubicin-induced cardiotoxicity in patients with lung cancer. Fourteen adult patients with normal resting cardiac function received 50-70 mg/m² of doxorubicin at regular intervals (N=7), or doxorubicin plus 100 mg/day of PO CoQ, beginning 3-5 days before the first dose of doxorubicin and continuing until therapy was completed (N=7). After a total cumulative dose of 600 mg/m², 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. In patients receiving 600 mg/m² of doxorubicin along with CoQ, cardiac function remained unchanged from that measured before therapy was started. Additionally, the seven patients taking CoQ 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 [58]. 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. The results of this study demonstrate that CoQ prevents doxorubicin-induced cardiomyopathy and that the total cumulative dose of doxorubicin can be escalated when CoQ is administered concurrently with the drug.

Other studies confirm these results. Cortes *et al.* [77, 78] measured systolic time intervals (STI: pre-ejection period/left ventricular ejection time) in 18 adults treated with 50 mg/m² doxorubicin (total cumulative dose of 200-500 mg/m²) plus vincristine and cyclophosphamide every 4 weeks. Eight of 10 patients receiving chemotherapy alone exhibited a progressive prolongation of STI (indicating depressed left ventricular function) with increasing cumulative doses of doxorubicin, and two patients developed congestive heart failure after 200 and 350 mg/m² of doxorubicin. In only 2 of 8 patients receiving chemotherapy plus 50 mg/day of PO CoQ was there an increase in STI detected, although one patient did develop CHF after 350 mg/m² of doxorubicin. Although these investigators used only a small dose of CoQ, the results suggest that CoQ reduces the cardiac toxicity of doxorubicin.

CoQ protection was also found in children treated with anthracyclines. 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 the anthracyclines (mean cumulative combined dose: 252 mg/m²) plus CoQ, 100 mg PO twice daily for the duration of the study. Echocardiographic evaluation was done before therapy started, after a cumulative anthracycline dose of 180 mg/m², and at the completion of therapy. Left ventricular function was reduced in both groups, although it occurred later and to a lesser degree in patients receiving CoQ [79].

Folkers *et al.* [80, 81] measured cardiac output before and during treatment of six adults with lung cancer who received doxorubicin every 3 to 4 weeks (3-5 infusions, total cumulative dose of 250-361 mg), four patients who received 3-4 infusions of doxorubicin (total cumulative dose of 215-355 mg) plus 60 mg/day PO CoQ, and five patients who received 2 infusions of doxorubicin (total cumulative dose of

145-175 mg) plus 60 mg/day PO CoQ. Patients receiving doxorubicin without CoQ exhibited a 25-40% reduction in cardiac output following the second (3 patients) or third (3 patients) drug infusion. In patients receiving CoQ, 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, but after the third and fourth infusions cardiac output was unchanged from that measured before treatment started. The remaining six patients exhibited no change in cardiac output throughout their treatment. Importantly, in the majority of patients in these studies cardiac output was maintained when CoQ was added during anthracycline treatment.

Okuma and Ota [82] randomized 80 cancer patients to receive doxorubicin or doxorubicin plus CoQ (90 mg/day PO beginning 1 week before chemotherapy was started and continuing until treatment was completed). Patients received 3-10 doxorubicin infusions and a total cumulative dose of 118-517 mg (doxorubicin only group) or 123-517 mg (doxorubicin plus CoQ). Patients receiving doxorubicin alone exhibited myocardial depression, with a significant depression of the QRS voltage beginning with the first infusion, and a significant prolongation of the Q-T interval starting after the fifth infusion. No significant change in the QRS voltage or the Q-T interval occurred in patients receiving CoQ.

Results of other studies suggest that CoQ also prevents the acute EKG changes that occur during therapy with doxorubicin. Takimoto *et al.* [83] investigated the impact of CoQ (90 mg/day PO) in a randomized study of 40 cancer patients who were treated with doxorubicin (50 mg/m²), cyclophosphamide, 5-fluorouracil plus radiation therapy. They found that administration of CoQ reduced the frequency and severity of changes in the QRS complex, S-T segment, and T-wave, and the frequency of arrhythmias. Tsubaki *et al.* [84] reported that IV infusion of 1 mg/kg/day of CoQ, for 4 days beginning 1 day before chemotherapy, reduced EKG changes induced by doxorubicin or daunorubicin. Yamamura [85] reported a similar effect of CoQ (30 mg/day PO) in patients being treated with doxorubicin.

CANCER-ASSOCIATED FATIGUE AND OXIDATIVE DAMAGE TO MITOCHONDRIA

Patients undergoing cytotoxic therapy frequently complain about the effects of therapy. Fatigue is usually the most common complaint, but other complaints include pain, nausea, vomiting, malaise, diarrhea, headaches, rashes, infections, and other more serious problems can occur, such as cardiomyopathy (discussed above), peripheral neuropathy, hepatotoxicity, pulmonary fibrosis, mucositis and other effects [86-88]. Most cancer patients reported fatigue associated with cancer therapy; however, only one-third of treating physicians recognized this problem [88]. Both physicians and patients complained more often of fatigue than pain, and most patients believed that fatigue associated with cancer therapy was untreatable [88, 89].

Cancer patients reported fatigue as a problem before receiving radio- or chemotherapy, and severe fatigue often occurs during or following cancer therapy [87-89]. In many studies fatigue was reported as the most troublesome and

disabling side effect during therapy [89-92], and it is often a significant reason why patients discontinue treatment [93]. Although fatigue is often the most commonly reported adverse symptom during cancer therapy, there has been little effort in controlling or reducing fatigue during cancer therapy [94]. 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 [95].

Although cancer patients often report fatigue, this is a rather common patient complaint associated with many illnesses. In fact, intractable or chronic fatigue lasting more than six months that is not reversed by sleep is the most common complaint of patients seeking general medical care [96-98]. It occurs naturally during aging and is also an important secondary condition in many clinical diagnoses [97, 98].

The phenomenon of fatigue has been defined as a multi-dimensional sensation, and recently attempts have been made to determine the extent of fatigue and its possible causes [99, 100]. Most patients understand fatigue as a loss of energy and inability to perform even simple tasks without exertion, and many medical conditions are associated with fatigue, including respiratory, coronary, musculoskeletal, and bowel conditions as well as infections and cancer [97-101].

At the cellular level fatigue is related to reductions in the efficiency of cellular energy systems that are found primarily in mitochondria [95, 101]. Damage to mitochondrial components, mainly by oxidation, can impair their ability to produce high-energy molecules, and oxidative stress caused by over-production of ROS/RNS is a major source of mitochondrial damage [2, 8, 12, 102-104]. Important targets of ROS/RNS damage are the phospholipid-containing membranes as well as mitochondrial DNA [90-92], and with aging and disease ROS/RNS damage accumulates and can eventually impair cellular functions [103-108].

During the development of chronic fatigue oxidative damage impairs mitochondrial function. For example, in chronic fatigue syndrome (CFS) patients there is evidence of oxidative damage to DNA and lipids [109, 110] as well as the presence of oxidized blood markers, such as methemoglobin, that are indicative of excess oxidative stress [111]. Evidence for oxidative damage to DNA and membrane lipids has been found in muscle biopsy samples obtained from CFS patients [112]. CFS patients 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 [113]. In addition to mitochondrial membranes, mitochondrial enzymes are also inactivated by peroxynitrite, and this could contribute to loss of mitochondrial function [114, 115].

MOLECULAR REPLACEMENT OF DAMAGED MEMBRANE COMPONENTS

The most sensitive targets of cellular ROS/RNS damage are the genetic apparatus and mitochondrial membranes [95, 101-104, 116]. In the case of membrane phospholipids oxidation modifies their structure, and this can affect lipid fluid-

ity, permeability and membrane function [117, 118]. One of the most important changes caused by accumulated ROS/RNS damage during aging and in chronic fatigue is loss of electron transport function, and this appears to be directly related to mitochondrial membrane lipid peroxidation [102], which induces permeability changes in mitochondria and loss of transmembrane potential, an essential requirement of mitochondrial oxidative phosphorylation [116].

Lipid Replacement Therapy [95, 101], a form of Molecular Replacement, along with antioxidants have been used to reverse ROS/RNS damage and increase mitochondrial function in certain clinical disorders, such as chronic fatigue, CFS and Fibromyalgia Syndrome [95, 119, 120]. Combined with antioxidant supplements, Lipid Replacement Therapy has proven to be an effective method to prevent ROS/RNS-associated changes and can reverse mitochondrial damage and loss of mitochondrial function [119, 120].

Molecular Replacement with unoxidized lipids given PO is possible because cellular lipids are in dynamic equilibrium in the body [101]. Orally ingested lipids diffuse to the gut epithelium and are bound and eventually transported into the blood and lymph using specific carrier lipoproteins and also by nonspecific partitioning and diffusion mechanisms [121, 122]. Within minutes, lipid molecules are transported from gut epithelial cells to endothelial cells, then excreted into and transported in the circulation bound to lipoproteins and blood cells where they are generally protected from oxidation [122, 123]. Once in the blood, specific lipoprotein carriers and red blood cells protect lipids throughout their transport and deposition onto specific cell membrane receptors where they can be taken into cells via endosomes and by diffusion [124]. Lipid transporters in the cytoplasm deliver specific lipids to cell organelles where they are taken in by specific transport proteins, in addition to partitioning and diffusion [125]. Damaged or oxidized lipids can be removed by a reverse process that is mediated by lipid transfer proteins and enzymes that recognize and degrade damaged lipids [125].

In addition to Lipid Replacement, dietary supplementation with antioxidants and some accessory molecules, such as zinc and certain vitamins, are important in maintaining cellular antioxidant and free-radical scavenging systems [121]. There are at least 40 micronutrients required in the human diet [126], and aging increases the need to supplement these to prevent age-associated damage to mitochondria and other cellular elements. Antioxidant use alone, however, may not be sufficient to maintain cellular components free of ROS/RNS damage [127]; thus Molecular Replacement is important in replacing ROS/RNS-damaged membrane lipids. During cancer chemotherapy Molecular Replacement is especially important, because excess oxidative stress modifies membranes and mitochondria to an extent far in excess of normal aging and disease (see above and following sections).

LIPID REPLACEMENT THERAPY: PRECLINICAL AND CLINICAL STUDIES

Molecular Replacement Therapy with unoxidized lipids and antioxidants PO results in replacement of damaged cellular and mitochondrial membrane phospholipids and other lipids that are essential structural and functional components

of all biological membranes [116-118]. One such lipid Molecular Replacement dietary supplement is NTFactor®, and this supplement has been used successfully in animal and clinical lipid replacement studies [101, 119, 120, 127, 128]. NTFactor's encapsulated lipids are protected from oxidation in the gut and can be absorbed and transported into tissues without undue damage. The composition of NTFactor is given elsewhere [101], but basically the active ingredient is a mixture of extracted membrane lipids (phospholipids, phosphoglycolipids, cardiolipids) whose composition is essentially the same as cellular membranes. Although the chemical composition of NTFactor is known, it is a lipid mixture not a single drug and thus its exact mechanism of action is not completely known.

NTFactor has also been used to reduce age-related damage in laboratory animals. In aged rodents, Seidman *et al.* [127] 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 [127]. Thus Molecular Replacement was successful in preventing age-associated hearing loss and reducing mitochondrial damage in rodents.

In clinical studies Lipid Molecular Replacement Therapy has been used to reduce fatigue and protect cellular and mitochondrial membranes from damage by ROS/RNS [119, 120]. A vitamin supplement mixture containing NTFactor has been used in a dietary Molecular Replacement study with severe chronic fatigued patients to reduce their fatigue [128]. Using the Piper Fatigue Scale [100] for measurement of fatigue we found that fatigue was reduced approximately 40.5% ($P < 0.0001$), from severe to moderate fatigue, after eight weeks of supplementation with NTFactor [128]. In more recent studies we examined the effects of NTFactor on fatigue in moderately and mildly fatigued subjects and to determine if their mitochondrial function, as measured by the transport and reduction of Rhodamine-123 and fatigue scores, improved with PO administration of NTFactor. Oral administration of NTFactor for 12 weeks resulted in a 35.5% reduction in fatigue, respectively ($P < 0.001$) [120]. 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 and mitochondrial function were intermediate between the initial starting values and those found after eight or 12 weeks on supplement [120]. The results indicate that in moderately to severely fatigued subjects dietary Lipid Molecular Replacement Therapy can significantly improve and even restore mitochondrial function and significantly improve fatigue. Similar findings were observed in CFS and Fibromyalgia Syndrome patients [119].

MOLECULAR REPLACEMENT/ANTIOXIDANT THERAPY FOR CANCER PATIENTS

Lipid Molecular Replacement Therapy plus antioxidants has also proven useful for reducing adverse effects in patients undergoing cancer chemotherapy. For example,

Table 4. Effects of Propax with NTFactor®, a Dietary Molecular Replacement Therapy Supplement, on the Adverse Effects of Chemotherapy in a Cross-Over Clinical Trial. †*

First Arm	Second Arm	Average % Patients on Test Supplement§		
		Improvement	No Change	Worsening
Placebo	Propax(+NTFactor)	57	22	21
Propax(+NTFactor)	Placebo	70	6	24

† Data from reference [129].

* The same regimen of 5-FU/methotrexate/leukovorin was used for colon, pancreatic or rectal cancers

§ The percent of patients reporting self adverse effects was averaged with the percent of patients with adverse effects reported by a research nurse.

Propax 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 [129]. Two studies were conducted by Colodny *et al.* [129] on advanced colon, pancreatic or rectal cancers receiving identical 5-FU/methotrexate/Leukovorin therapy on a 12-week schedule. In the 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. This quality of life evaluation was conducted by a research nurse, and it was determined that patients on Propax supplementation experienced 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 the open label part of the trial 81% of patients demonstrated an overall improvement in quality of life parameters while on chemotherapy. In the double-blinded, cross-over, placebo-controlled, randomized part of the study on advanced cancers the patients on Lipid Molecular Replacement Therapy showed improvements in signs/symptoms associated with chemotherapy but only in the arm of the trial where the supplement was administered [129]. Lipid Molecular Replacement Therapy with Propax resulted in improvement from 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 (Table 4). This preliminary clinical trial demonstrated that usefulness of Lipid Molecular Replacement Therapy and antioxidants given during chemotherapy.

SUMMARY

Molecular Replacement Therapy or replacement of lipids and cofactors during cancer chemotherapy reduces the adverse effects of cytotoxic therapy and limits oxidative stress-related damage to normal cellular structures. Oral Molecular Replacement supplements can be used to replace normal cellular constituents that are damaged as a therapeutic consequence of excess oxidative stress. Molecular Replacement Therapy does not diminish the anti-cancer cell therapeutic properties of chemotherapy drugs, but it does help protect normal cells and thus increases the therapeutic ratio. Molecular Replacement Therapy is a cost-effective and safe method to reduce the adverse chronic and acute effects of cancer chemotherapy and increase cancer therapeutic ratios.

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