

# Coenzyme Q10 for Prevention of Anthracycline-Induced Cardiotoxicity

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Preclinical and clinical studies suggest that anthracycline-induced cardiotoxicity can be prevented by administering coenzyme Q10 during cancer chemotherapy that includes drugs such as doxorubicin and daunorubicin. Studies further suggest that coenzyme Q10 does not interfere with the antineoplastic action of anthracyclines and might even enhance their anticancer effects. Preventing cardiotoxicity might allow for escalation of the anthracycline dose, which would further enhance the anticancer effects. Based on clinical investigation, although limited, a cumulative dose of doxorubicin of up to 900 mg/m<sup>2</sup>, and possibly higher, can be administered safely during chemotherapy as long as coenzyme Q10 is administered concurrently. The etiology of the dose-limiting cardiomyopathy that is induced by anthracyclines can be explained by irreversible damage to heart cell mitochondria, which differ from mitochondria of other cells in that they possess a unique enzyme on the inner mitochondrial membrane. This enzyme reduces anthracyclines to their semiquinones, resulting in severe oxidative stress, disruption of mitochondrial energetics, and irreversible damage to mitochondrial DNA. Damage to mitochondrial DNA blocks the regenerative capability of the organelle and ultimately leads to apoptosis or necrosis of myocytes. Coenzyme Q10, an essential component of the electron transport system and a potent intracellular antioxidant, appears to prevent damage to the mitochondria of the heart, thus preventing the development of anthracycline-induced cardiomyopathy.

**Keywords:** anthracyclines; antioxidants; breast cancer; cancer; doxorubicin; cardiotoxicity; coenzyme Q10; mitochondria

The anthracycline antibiotics are important antineoplastic agents. This class of drugs includes the naturally occurring doxorubicin and daunorubicin that are produced by species of the fungus *Streptomyces* as well as the synthetic derivatives epirubicin and idarubicin that differ only slightly in structure from the parent compounds (Figure 1). These drugs have a broad range of clinical applications for adults and children, with demonstrated efficacy for the treatment of hematological cancers (leukemias and lymphomas) as

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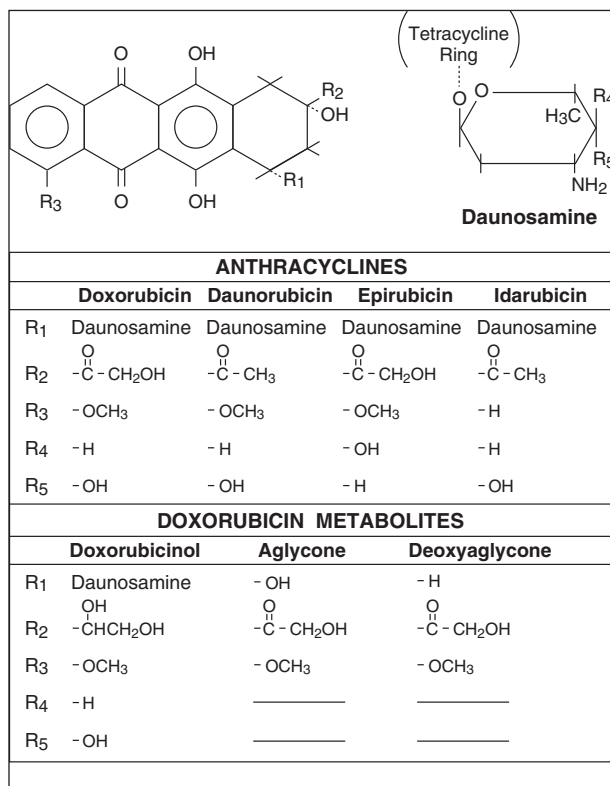


Figure 1 The structures of anthracyclines and doxorubicin metabolites.

well as a variety of solid malignancies (carcinomas and sarcomas). Thus, these agents are the cornerstone of many chemotherapy regimens and possibly the most important component of some such as the use of doxorubicin for treatment of breast cancer. However, although there is a dose-response relation for the anthracyclines in the treatment of cancer, there is also a dose-related cardiac toxicity that occurs with all drugs of this class.

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Anthracyclines produce acute and chronic cardiotoxicity. Although acute cardiotoxicity, manifest by various electrocardiographic (EKG) changes and depression of myocardial contractility, is commonly seen in patients during the first 24 hours after drug infusion, the effects are transient and resolve spontaneously.<sup>1,2</sup> Laboratory animals exhibit manifestations of acute cardiotoxicity that are similar to those of humans.<sup>3,8</sup>

Of far greater concern than acute toxicity is the chronic cardiotoxic effect of anthracyclines, with patients exhibiting irreversible cardiomyopathic changes and congestive heart failure (CHF) that does not respond to conventional pharmacological therapies. Doxorubicin, the most widely used anthracycline, exhibits greater cardiotoxicity than the other drugs of this class. Although the total cumulative dose of doxorubicin that is associated with the development of cardiomyopathy varies widely (from 75 to 1095 mg/m<sup>2</sup>),<sup>9</sup> the median dose at which this toxicity is observed was reported to be 390 mg/m<sup>2</sup> in 88 cases in a series of 4018 patients (overall incidence of 2.2%).<sup>10</sup> The dose-related risk was 3% with a dose of 400 mg/m<sup>2</sup>, 7% with 550 mg/m<sup>2</sup>, 18% with 700 mg/m<sup>2</sup>, and 50% with 950 mg/m<sup>2</sup>. The slope of the dose-toxicity curve increases at about 550 mg/m<sup>2</sup>.

Cardiomyopathy is reported to develop between 0 and 231 days (median 23 days) following the final dose of doxorubicin<sup>10</sup> and between 9 and 192 days (median 60 days) following the final dose of daunorubicin.<sup>11</sup> However, delayed development of cardiotoxicity of up to 20 years following therapy has been reported,<sup>12,14</sup> suggesting that processes necessary for sustaining the integrity of the cardiac myocyte are damaged during therapy. Although cardiac cells do not undergo growth and division, components of many subcellular structures and organelles must be continually regenerated to maintain myocyte function. An important example is maintenance of the functional integrity of mitochondria, for which components of the energy-generating system (electron transport and oxidative phosphorylation) must be renewed during the life of the myocyte.

Damage to the mitochondria of cardiac cells is one of the earliest and most prominent histological findings of anthracycline-induced cardiomyopathy in humans<sup>2,15-18</sup> and laboratory animals.<sup>19-31</sup> Damage to cardiac mitochondria but not to mitochondria of other tissues<sup>31</sup> may explain why anthracyclines selectively damage the heart. Since the spectrum of myocyte damage in rabbits,<sup>19-24</sup> rats,<sup>25,26</sup> and mice<sup>27-31</sup> is similar to that in humans,<sup>2,15-18</sup> laboratory animals provide a model for investigating cardiomyopathy. Although interference with mitochondrial energetics may account for acute cardiotoxicity, cardiomyopathy may

result from damage to processes that maintain the long-term integrity of cardiac mitochondria. Other effects of anthracyclines on the heart that are seen histologically, including damage to contractile elements and impairment of intracellular processes such as calcium homeostasis,<sup>32</sup> may be explained by progressive deterioration of mitochondrial bioenergetics.

### **Anthracyclines and Oxidative Stress**

A prominent hypothesis regarding the etiology of anthracycline-induced cardiotoxicity is that cardiac damage is caused by oxidative stress, that is, the generation of reactive oxygen species (ROS) that include free radicals such as superoxide and hydroxyl radicals and nonradical oxygen species such as hydrogen peroxide. Since lipids are a primary target of ROS, oxidative stress is associated with the generation of lipid peroxidation products (primary products such as peroxy and alkoxy radicals) and numerous aldehydes (malondialdehyde [MDA], 4-hydroxyalkenals, etc) that are secondary products of lipid peroxidation.<sup>33</sup> Administering doxorubicin to laboratory animals<sup>34-41</sup> and humans<sup>43-48</sup> results in an elevation of plasma and tissue ROS and products of lipid peroxidation and a decrease in plasma and tissue antioxidant levels. The level of doxorubicin-induced oxidative stress is up to 10 times greater in the heart than it is in other tissues such as the liver, kidney, and spleen.<sup>34,35,41</sup> The high level of oxidative stress generated by anthracyclines is accounted for by structural characteristics that allow the drugs to participate in electron-accepting and -donating reactions.

Doxorubicin, as other anthracyclines, possesses a unique hexose sugar, daunosamine, attached to a tetracycline structure containing adjacent quinone and hydroquinone moieties (Figure 1) that permits it to accept an electron and be reduced to its semiquinone.<sup>49</sup> The semiquinone readily donates its electron to molecular oxygen to form the superoxide radical. Although superoxide is not highly toxic, superoxide dismutase generates hydrogen peroxide, which, in the presence of reduced iron or copper, forms highly toxic hydroxyl radicals via a Fenton or Haber-Weiss reaction.<sup>49</sup> Many intracellular enzymes can reduce doxorubicin, including cytosolic xanthine oxidase<sup>50</sup> and microsomal nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P450 reductase,<sup>50-53</sup> which is a primary site for ROS generation from a chemically diverse group of compounds including many antineoplastic agents. Although the NADPH-dependent microsomal enzyme is present in all tissues including the heart<sup>51</sup> and liver,<sup>52,53</sup> cardiac cells, by means of a mitochondrial nicotinamide adenine dinucleotide (NADH) dehydrogenase that is not

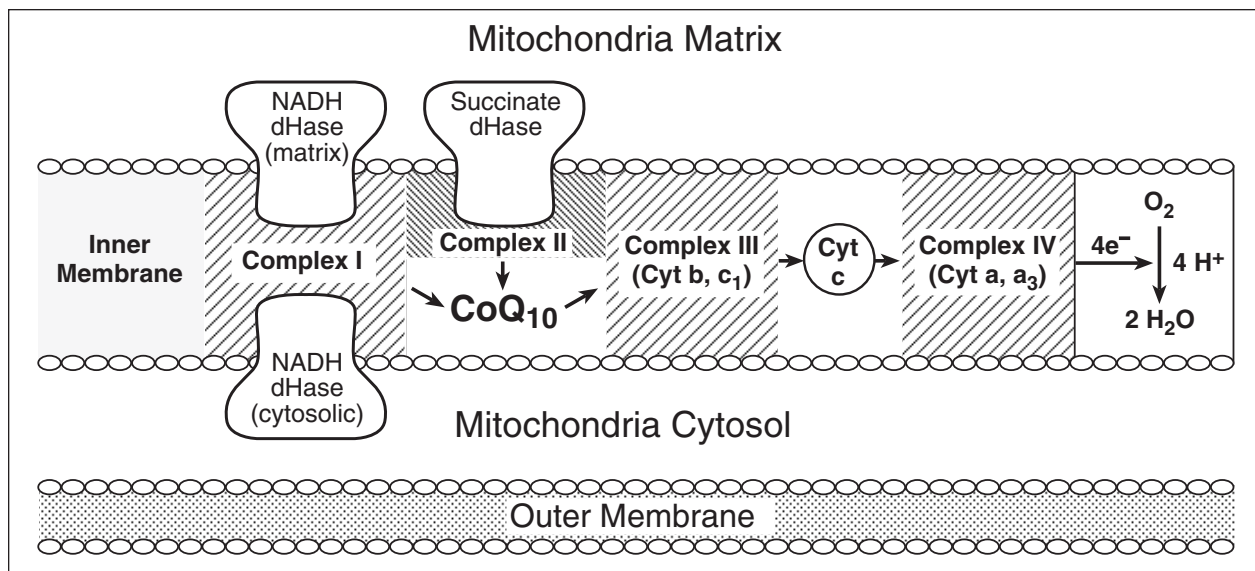


Figure 2 Structure of heart cell mitochondria with the components of the electron transport system. NADH dHase = nicotinamide adenine dinucleotide dehydrogenase; succinate dHase = succinate dehydrogenase; Cyt b,c<sub>1</sub> = cytochrome b and cytochrome c<sub>1</sub>; Cyt c = cytochrome c; Cyt a,a<sub>3</sub> = cytochrome a and cytochrome a<sub>3</sub>; CoQ<sub>10</sub> = coenzyme Q10. The arrows indicate the flow of electrons (e<sup>-</sup>) in the electron transport system.

present in other types of cells, generate very high levels of free radicals in the presence of doxorubicin.<sup>34,35,41</sup> This explains the high degree of oxidative stress that the drug causes in cardiac mitochondria,<sup>54</sup> cultured heart cells,<sup>55</sup> and the isolated-perfused heart.<sup>56</sup> The unique structure of the electron transport system (ETS) of cardiac mitochondria accounts for the high level of oxidative stress generated by doxorubicin and for the development of cardiomyopathy.

### Cardiac Mitochondria and Their Role in the Generation of Doxorubicin-Induced Oxidative Stress and Cardiotoxicity

Energy production by mitochondria begins with the tricarboxylic acid (TCA) cycle that provides reducing equivalents to the ETS. The ETS is coupled to oxidative phosphorylation for the generation of adenosine 5'-triphosphate (ATP). The components of the TCA cycle, except for succinate dehydrogenase, which is part of the ETS (complex II), reside within the mitochondrial matrix (Figure 2). The inner membrane contains the components of the ETS and is studded with spheres, projecting into the matrix, that contain the ATP-synthesizing apparatus. Between the inner and outer membranes is the intermembrane space or mitochondrial cytosol. All hydrophilic molecules that enter or pass through the inner membrane require transport systems. In contrast to the inner membrane, the outer membrane is freely permeable to all molecules with molecular weights less than 10 000 d.

Electrons are transferred by the ETS from complex I (NADH dehydrogenase) and complex II (succinate

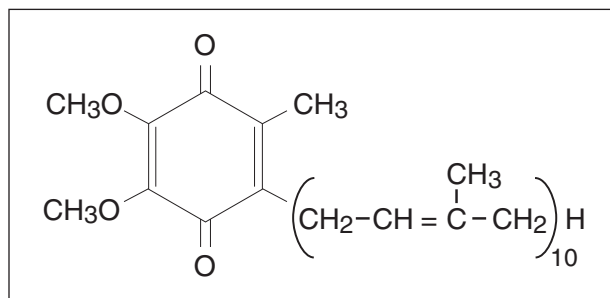


Figure 3 The structure of coenzyme Q10.

dehydrogenase) to coenzyme Q10 (CoQ), which possesses a quinone structure (Figure 3) that allows it to function in electron-accepting and -donating reactions. Electrons are then sequentially transferred to complex III, cytochrome c, and complex IV. Finally, 4 electrons are transferred to oxygen, and water is formed. The enzymatically active components of the ETS in all mitochondria are closely associated with the matrix surface of the inner membrane. In the mitochondria of organs other than the heart, and of tumors as well, complex I and complex II, which initiate the transfer of reducing equivalents that they receive from the matrix, do not have access to nor can they react with molecules in the mitochondrial cytosol.<sup>57-59</sup> However, the mitochondria of cardiac cells are unique in that they possess an NADH dehydrogenase on the outer (cytosolic or intermembranous) surface of the inner membrane in addition to the NADH dehydrogenase that faces the matrix.<sup>60,61</sup> This cytosolic NADH dehydrogenase is

associated with complex I and is able to introduce reducing equivalents from the mitochondrial cytosol into the ETS.<sup>57,61</sup> This enzyme is able to react not only with NADH but also with other molecules that gain access to the mitochondrial cytosol. Because of its access to the mitochondrial cytosol, this NADH dehydrogenase appears to be responsible for anthracycline-associated cardiotoxicity.

Doxorubicin, with a molecular weight of less than 600 d, readily passes through the outer membrane to enter the mitochondrial cytosol. However, because it is hydrophilic, it cannot penetrate the lipoidal inner membrane to reach the matrix NADH dehydrogenase. Thus, doxorubicin cannot be reduced by the ETS of most types of cells, such as those of liver, kidney, and tumors.<sup>59-61</sup> In contrast, the cytosolic NADH dehydrogenase of cardiac mitochondria reduces doxorubicin to its semiquinone,<sup>62,65</sup> which can donate an electron to molecular oxygen resulting in oxidative stress. In addition, the semiquinone undergoes autooxidation to form the fully reduced dihydroquinone. This destabilizes the molecule, resulting in cleavage of the sugar moiety and formation of doxorubicin aglycones (Figure 1),<sup>49,65</sup> which are major metabolites of doxorubicin. The highly lipophilic aglycones penetrate the inner membrane and serve as electron-acceptor and -donor molecules in place of CoQ. Although doxorubicin aglycones can inhibit the enzymes of complex I and II, this effect occurs only at concentrations (>100  $\mu$ M) that far exceed those that are reached clinically.<sup>66</sup> Thus, the main action of the aglycones on the ETS is displacement of CoQ, which is not covalently or otherwise tightly bound within the inner membrane. Displacement of CoQ from the mitochondrial inner membrane, as well as from other intracellular membranes where it serves as an antioxidant, is evident by the acute rise in the plasma CoQ level when doxorubicin is administered to patients during chemotherapy,<sup>67</sup> and by the marked decrease of the CoQ content of cardiac and skeletal muscle following chemotherapy with doxorubicin.<sup>68</sup>

Once doxorubicin aglycones displace CoQ from the mitochondrial inner membrane, they serve as electron acceptors from complex I and II. However, instead of transferring electrons to complex III, the aglycones transfer electrons to molecular oxygen, resulting in the formation of superoxide radicals.<sup>65</sup> Mitochondrial superoxide dismutase then generates hydrogen peroxide, and in the presence of reduced iron that is plentiful in mitochondria, high levels of hydroxyl radicals are produced. Thus, cardiac cells are exposed to very high levels of oxidative stress arising both from the ETS and from the doxorubicin semiquinone in the mitochondrial cytosol. In

addition to the energy-generating capacity of cardiac mitochondria being acutely interrupted by displacement of CoQ from the ETS, which likely accounts for the changes in the EKG and myocardial contractility seen shortly after administration of doxorubicin, the high level of oxidative stress can create additional cellular damage. Although this oxidative stress may further impair mitochondrial energetics acutely, many cellular targets, such as DNA, may sustain free radical damage.

Oxidative damage to DNA, as shown by the excretion of thymine oxidative products, has been demonstrated in doxorubicin-treated patients.<sup>48</sup> However, in heart cells, doxorubicin induces far greater damage to mitochondrial DNA (mtDNA) than to nuclear DNA (nDNA)<sup>39,69,70</sup> due to the greater susceptibility of mtDNA to oxidative damage<sup>71</sup> and to the high level of oxidative stress that the drug creates in cardiac mitochondria. The degree of mtDNA oxidation in heart cells is far greater than it is in liver cells,<sup>70</sup> demonstrating the cardioselective impact of doxorubicin. Although mtDNA repair does occur, it is far less efficient than nDNA repair.<sup>72</sup> Thus, doxorubicin-induced mtDNA oxidation, which is cumulative following repeated doses of the drug<sup>70</sup> and continues for several weeks following the last dose,<sup>39</sup> results in damage to the mitochondrial genome. Much of this damage, such as significant mtDNA deletions that are seen following treatment with doxorubicin, is not repairable.<sup>40</sup> Damage to mtDNA by doxorubicin may explain why some individuals do not manifest signs and symptoms of cardiomyopathy for months or years following chemotherapy. Since mtDNA encodes for several components of the ETS and for mitochondrial ribosomal and transfer RNAs, suppression of mitochondrial gene expression due to doxorubicin-induced damage, which has been demonstrated,<sup>31,73</sup> would result in the inability of mitochondria to sustain their regenerative processes. This would be expected to result in a delayed onset of cardiac dysfunction. Damage to mtDNA that results in doxorubicin-induced cardiomyopathy may be analogous to the mtDNA damage that is felt to be an important contributory factor in idiopathic cardiomyopathy.<sup>74</sup>

## CoQ

CoQ (ubiquinone) is an integral part of the energy-generating system of all animals and plants and most aerobic microorganisms.<sup>75,76</sup> It is synthesized by all organisms that require it, with the aromatic portion of the molecule being derived from tyrosine through a series of 8 reactions.<sup>77</sup> The concentration of CoQ in different mammalian tissues reflects the cellular content of mitochondria. In the heart, the CoQ concen-

tration is 5 times that of the liver and approximately 10 times that of the kidney, pancreas, and spleen.<sup>76</sup> The concentration of CoQ in skeletal muscle is only 5% of that found in the heart.<sup>76</sup>

The numerical designation in CoQ (eg, the 10 in coenzyme Q10) refers to the number of isoprene units in the side chain (Figure 3). The number of isoprene units in the CoQ of most mammals is 10, although it is frequently less than 10 in other animal species and plants. The isoprene side chain gives CoQ a highly lipophilic property, which allows it to diffuse rapidly into membrane phospholipid bilayers.<sup>78</sup> Thus, in addition to its essential position in the ETS of the inner mitochondrial membrane, it is also localized in many other cellular membranes including the Golgi apparatus, the endoplasmic reticulum, lysosomes, the outer mitochondrial membrane, and the plasma membrane.<sup>78,79</sup> It is within these membranes that CoQ acts as a powerful antioxidant, scavenging lipid radicals and inhibiting lipid peroxidation.<sup>78-81</sup> As a peroxy scavenger, CoQ is somewhat more potent than  $\alpha$ -tocopherol (vitamin E), and CoQ plus  $\alpha$ -tocopherol have additive antioxidant effects.<sup>82</sup> CoQ also functions to maintain  $\alpha$ -tocopherol in the reduced state in membranes and to maintain ascorbate (vitamin C) in the reduced state both inside and outside the cell.<sup>83</sup> Within the inner mitochondrial membrane, CoQ is a requirement for the enzymatic recycling of  $\alpha$ -tocopherol.<sup>84</sup> CoQ is probably the most important antioxidant in cardiac myocytes,<sup>85</sup> where it has a protective effect against oxidative damage to mitochondrial protein and mtDNA.<sup>86</sup> CoQ is also an important antioxidant in plasma, where it protects low-density lipoproteins (LDL) from lipid peroxidation.<sup>87,88</sup> Within LDL, CoQ is a more efficient antioxidant than  $\alpha$ -tocopherol, lycopene, or  $\beta$ -carotene.<sup>89</sup> CoQ administered orally (PO) to human volunteers prevents oxidative stress induced by feeding polyunsaturated fatty acids.<sup>90</sup>

All tissues synthesize CoQ in mitochondria and in most intracellular membranes such as the endoplasmic reticulum,<sup>91</sup> although CoQ is also bioavailable through the diet. Following PO administration to rats, CoQ is taken up primarily by the liver.<sup>92,93</sup> The amount taken up by the adrenal glands (CoQ/g of tissue) is about half that of liver, whereas smaller amounts are taken up by the heart, lung, kidney, and spleen.<sup>92,93</sup> CoQ administered PO to humans is also taken up in small amounts by the heart.<sup>94</sup> When it is administered parenterally to rats, CoQ is incorporated into the inner membrane of heart mitochondria.<sup>95</sup> In adult humans, the usual daily intake of CoQ from food is estimated to be 3 to 5 mg<sup>96</sup> to as high as 20 mg,<sup>97</sup> with approximately two thirds of the total being

derived from animal-source protein.<sup>96</sup> The usual plasma concentration of CoQ in healthy adults is 0.6 to 1.0 mg/L (0.7-1.2  $\mu$ M).<sup>87,90,96,98-100</sup> Individuals with cancer, however, have lower plasma levels of CoQ, with the lowest level being found in women with breast cancer.<sup>101</sup> In healthy adult volunteers, a single PO dose of 30 mg (0.4 mg/kg) CoQ raises the peak plasma level by 35% to 50%,<sup>96,99</sup> whereas single PO doses of 100, 200, and 300 mg increase the peak plasma level by 80% to 130%,<sup>87,102</sup> 150%,<sup>87</sup> and 190%,<sup>87</sup> respectively. However, daily PO dosing for several days is necessary for the plasma level of CoQ to reach a plateau. Thus, repeated daily doses of 90 mg CoQ for 2 weeks raises the plasma level by 200%,<sup>90</sup> and a daily dose of 300 mg for 11 days raises the level by 300% to 400%,<sup>87</sup> although it appears to take more than 2 weeks of daily dosing for the plasma CoQ level to reach a plateau.<sup>90,98</sup> Following a single PO dose of CoQ, the plasma level exhibits a 2-peak pattern, with an initial peak at 6 hours and a second lower peak at 24 hours.<sup>102-105</sup> The second peak has been attributed to redistribution from the liver<sup>105</sup> and to entero-hepatic recycling.<sup>104</sup> Absorption of CoQ is enhanced when it is taken with a meal or when taken as an oil-based formulation instead of a formulation with inert substances.<sup>96,99,102</sup>

### Anthracycline Pharmacokinetics

The mean plasma level of doxorubicin measured 5 minutes following a 30 mg/m<sup>2</sup> intravenous (IV) bolus injection is 2.5 to 2.8  $\mu$ M.<sup>106,107</sup> The mean plasma level measured 5 minutes following a 15-minute IV infusion of 75 mg/m<sup>2</sup> doxorubicin is 5  $\mu$ M,<sup>108</sup> whereas 10 minutes following a 30-minute IV infusion of 75 mg/m<sup>2</sup>, the mean plasma level is 3.5  $\mu$ M.<sup>109</sup> IV doses of 100, 125, and 150 mg/m<sup>2</sup> of doxorubicin infused over 30 minutes result in mean plasma levels that are proportionately higher than that achieved with the 75-mg/m<sup>2</sup> dose.<sup>109</sup> One hour following an IV injection of doxorubicin, the plasma level falls to 0.05 to 0.1  $\mu$ M with a 30-mg/m<sup>2</sup> dose<sup>106,107</sup> and to 0.2 to 0.3  $\mu$ M with a 75-mg/m<sup>2</sup> dose.<sup>108,109</sup> The plasma concentration of daunorubicin 5 minutes after a 5-minute IV infusion of 45 mg/m<sup>2</sup> is 0.4  $\mu$ M, and 1 hour later, the concentration is approximately 0.05  $\mu$ M.<sup>110</sup> A 96-hour IV infusion of 9 mg/m<sup>2</sup>/24 h of doxorubicin (total of 36 mg/m<sup>2</sup>)<sup>106</sup> or a 72-hour IV infusion of 15 mg/m<sup>2</sup>/24 h of daunorubicin (total 45 mg/m<sup>2</sup>)<sup>110</sup> results in a steady-state drug concentration in plasma of approximately 30 nm that is reached 24 to 48 hours after the infusion is started. Plasma disappearance of doxorubicin and daunorubicin follows a bi- or triexponential function with terminal elimination half-lives of about 30 hours<sup>106-109,111</sup> and 55 hours,<sup>112</sup> respectively.

At the end of a 96-hour IV infusion of 9 mg/m<sup>2</sup>/24 h of doxorubicin, the mean drug concentration in bone marrow cells and circulating nucleated blood cells of patients with multiple myeloma is 5.1 μM and 5.0 μM, respectively, which is nearly 200 times higher than the corresponding plasma level.<sup>106</sup> Although the concentration of doxorubicin in plasma remained unchanged during the final 48 hours of the infusion, the concentration in both bone marrow and nucleated blood cells continued to rise until the end of the infusion. In patients with leukemia, the mean leukemia cell concentration of doxorubicin 5 minutes following a 30-mg/m<sup>2</sup> IV bolus injection is 9.9 μM (approximately 4 times the corresponding plasma level).<sup>106</sup> The elimination half-life of cellular doxorubicin was 87 hours in nucleated blood cells and 116 hours in leukemia cells. In leukemia cells, the concentration of daunorubicin after an IV bolus injection or 72-hour IV infusion of 45 mg/m<sup>2</sup> is 29 μM and 18 μM, respectively, and the cellular half-life is 13 hours.<sup>110</sup>

The concentration of doxorubicin in several types of solid tumors, removed from patients who received the drug just prior to surgery, varied from 0.2 to 16 μM at 1.5 to 5 hours following an IV bolus injection of 30 mg/m<sup>2</sup> or 30 minutes following an IV bolus injection of 25 mg/m<sup>2</sup>.<sup>113,114</sup>

Intracellular anthracyclines are localized primarily in the nucleus of drug-sensitive cancer cells,<sup>115-119</sup> and for doxorubicin, more than 99.7% of the drug within the nucleus is bound to DNA.<sup>120</sup> In contrast to drug-sensitive cancer cells, anthracycline-resistant cancer cells take up far less drug, and that which is taken up is localized primarily in the cytoplasm.<sup>115-118</sup>

Anthracyclines are extensively metabolized in humans. The metabolites of doxorubicin are doxorubicinol, formed by reduction of the carbonyl group, the aglycones and deoxyaglycones of doxorubicin and doxorubicinol, and the sulfate and glucuronide conjugation (at R3 in Figure 1) products of demethyldeoxydoxorubicinol aglycone.<sup>121</sup> Daunorubicin also undergoes reduction of its carbonyl group to form its alcohol derivative daunorubicinol.<sup>110</sup> The primary metabolite of both doxorubicin and daunorubicin is the corresponding alcohol. The plasma concentrations of these 2 metabolites remain significantly above those of their parent drugs beginning shortly after drug administration and continuing for up to 216 hours.<sup>110,111</sup> The cellular level of daunorubicin and daunorubicinol remain nearly the same for up to 192 hours following either an IV bolus dose or a 72-hour IV infusion of daunorubicin.<sup>110</sup> The combined plasma concentration of doxorubicin aglycones is approximately double that of the parent

drug for the first 48 hours after drug injection.<sup>111</sup> Doxorubicinol, which retains the sugar moiety, binds DNA and exhibits antineoplastic activity.<sup>111,121,122</sup> The aglycones do not bind DNA, nor do they exhibit antineoplastic activity.

## CoQ and Anthracycline Cardiotoxicity

### Studies with Cell-Free Systems

Doxorubicin, doxorubicin aglycone, and daunorubicin aglycone inhibit NADH dehydrogenase of complex I and succinate dehydrogenase of complex II that have been isolated from beef heart mitochondria.<sup>66,123</sup> Doxorubicin aglycone is 5 times more inhibitory than doxorubicin. CoQ is able to fully reverse the enzyme inhibition by doxorubicin at a molar ratio of 3:1 (CoQ:doxorubicin), although it has no effect on enzyme inhibition by doxorubicin aglycone even at a molar ratio of 100:1 (CoQ:aglycone). However, the drug concentrations needed to inhibit enzyme activity (ID<sub>50</sub> of approximately 900 μM for doxorubicin and 180 μM for doxorubicin aglycone) are far in excess of the intracellular concentrations achieved clinically, and daunorubicin aglycone, at a concentration of 900 μM, did not inhibit the activity of succinate dehydrogenase. These results suggest that enzyme inhibition of complex I and II does not play a role in the cardiotoxicity of anthracyclines.

Doxorubicin added to isolated rat heart mitochondria inhibits NADH dehydrogenase and succinate dehydrogenase activity measured by the rate of substrate oxidation, although the ID<sub>50</sub> for inhibition is in the range of 200 to 500 μM.<sup>124,125</sup> The drug effect is only minimally affected by CoQ at a molar ratio of 1:1, and even at a ratio of 15:1 (CoQ:doxorubicin), inhibition of succinate oxidation is only partially prevented. NADH dehydrogenase and succinate dehydrogenase activities in beef heart mitochondria are less sensitive to inhibition by doxorubicin (ID<sub>50</sub> of 500 μM and 800 μM, respectively), although the inhibition can be prevented by adding CoQ at a molar ratio of 4:1 (CoQ:doxorubicin).<sup>126</sup> A comparable degree of enzyme inhibition by daunorubicin requires doses that are approximately 4 times higher than that of doxorubicin. The observed lack of enzyme inhibition by the drugs, except at very high concentrations, is expected since the drugs cannot penetrate the inner membrane to disrupt the ETS. The results further indicate that aglycone formation does not occur in these mitochondrial preparations. If aglycones were formed, significant inhibition of oxygen consumption would be expected. However, manometric experiments such as these allow for only a very short exposure time (a few minutes) to the drug. Thus, there may

have been insufficient time for formation of aglycones and for their penetration into the inner membrane.

Doxorubicin induces lipid peroxidation (MDA formation) in isolated beef heart mitochondria.<sup>54</sup> The addition of NADH as an electron donor markedly increases MDA formation. Since doxorubicin and NADH do not penetrate the mitochondrial inner membrane, these results demonstrate that the cytosolic NADH dehydrogenase reduces doxorubicin. The addition of 40  $\mu\text{M}$  CoQ reduces MDA production by about 50%. Several mechanisms could explain the effect of CoQ, including (1) competition between CoQ and doxorubicin for the cytosolic NADH dehydrogenase, which would decrease the amount of drug that is reduced; (2) oxidation of doxorubicin semiquinone by CoQ, which would prevent formation of superoxide radicals and doxorubicin aglycones; or (3) quenching by CoQ of free radicals that are generated by doxorubicin semiquinone, thus preventing lipid peroxidation.

Mitochondria isolated from the heart, liver, and kidneys of rats injected with a relatively high intraperitoneal (IP) dose of doxorubicin (3.5 mg/kg/d for 8 days) exhibit reduced activity of NADH and succinate dehydrogenases.<sup>124</sup> The effect on heart mitochondria is 2 to 3 times greater than the effect on mitochondria of the other tissues. These results are consistent with damage to the mitochondrial energy-generating systems of all 3 tissues, with the greater impact on cardiac mitochondria being due to the cytosolic NADH dehydrogenase. CoQ added to the mitochondrial preparations at a concentration of 0.5 mM CoQ (approximately 500 times the normal plasma concentration) did not reverse the impairment of mitochondrial energetics. Thus, although CoQ appears to prevent mitochondrial damage when sufficient doses are administered before doxorubicin administration (see below), it does not correct doxorubicin-induced damage to heart mitochondria once the damage has occurred. This suggests that the drug creates irreversible damage to mitochondria, possibly via the high level of oxidative stress that it induces in all cells, especially in cardiac cells.

In mitochondria isolated from rat liver and beef heart, doxorubicin inhibits the synthesis of CoQ.<sup>127</sup> However, 50% inhibition requires a doxorubicin concentration of nearly 1 mM. Thus, inhibition of CoQ synthesis by mitochondria is unlikely to play a role in doxorubicin toxicity.

#### **Studies With Cell Cultures/Suspensions and Tissue/Organ Preparations**

In cultured mouse myocardial cells, 3.5  $\mu\text{M}$  doxorubicin reduced the percentage of beating cells by 28%, 58%, and 95% at 24 hours, 48 hours, and 72

hours, respectively.<sup>55,128,129</sup> The beating rate of cells that remained beating was reduced by 25%, 65%, and 95% at the same time points. The addition of 120  $\mu\text{M}$  CoQ with doxorubicin provided significant protection from the doxorubicin-induced decrease of cell beating. Doxorubicin increased the MDA concentration to 50% above that of a control culture (without doxorubicin) after 48 hours. CoQ, 120  $\mu\text{M}$ , added with doxorubicin maintained the MDA level at the level of the control culture without affecting the uptake of doxorubicin by myocardial cells.<sup>128</sup> In contrast to the effects of CoQ, 120  $\mu\text{M}$   $\alpha$ -tocopherol reduced the MDA level of the doxorubicin-treated culture to below that of the control culture but did not prevent the impact of doxorubicin on cell beating or the beating rate.<sup>55,129</sup> These results suggest that beat inhibition by doxorubicin, which may reflect acute cardiotoxicity, is not due to drug-induced oxidative stress and that CoQ protects cardiac cells by blocking the intracellular effects of the drug (aglycone formation and interference with mitochondrial energetics by the aglycones).

CoQ or  $\alpha$ -tocopherol, in an equimolar concentration to that of doxorubicin, completely inhibit oxidative stress induced by the drug in Ehrlich ascites carcinoma cells.<sup>130</sup> However, Ehrlich ascites cells with or without doxorubicin develop MDA levels that are less than 10% of the levels that myocardial cells develop.<sup>55,129</sup> Others have also observed that cancer cells and tumors generate far lower levels of lipid peroxidation than do normal cells and tissues.<sup>131-135</sup> Several mechanisms account for the lower level of oxidative stress in cancer cells compared to that of normal cells, including the following: (1) tumor cell mitochondria are poorly developed and 50% to 80% fewer than in normal cells (cancer cells depend far more on glycolytic pathways for ATP production)<sup>72,136</sup>; (2) tumor cells have relatively higher antioxidant levels, such as a higher ratio of  $\alpha$ -tocopherol to peroxidizable moieties (methylene groups) of the polyunsaturated fatty acids in their biological membranes<sup>134,135,137</sup>; and (3) tumor cells have lower levels of the NADPH-cytochrome P450 electron transport system,<sup>133-135,138</sup> which is a primary site of doxorubicin reduction.<sup>50-53</sup>

In the isolated-perfused rat heart, 8.5 and 17  $\mu\text{M}$  doxorubicin reduced contractile tension by 35% and 60%, respectively, after a 60-minute perfusion.<sup>7</sup> The reduced tension was sustained during a 30-minute doxorubicin washout, as was the tissue concentration of doxorubicin. At 17  $\mu\text{M}$ , doxorubicin reduced the heart tissue levels of NAD<sup>+</sup>, NADH, ATP, adenosine 5'-diphosphate, adenosine 5'-monophosphate, and creatine phosphate. In rats given IP CoQ, 15 mg/kg/d for 7 days prior to heart excision, the adverse effect of doxorubicin on contractile tension and levels of high-

energy phosphates and metabolic intermediates was significantly reduced. This suggests that CoQ pretreatment enhances myocardial content of CoQ and reduces the acute cardiotoxic effects of doxorubicin. In addition, CoQ pretreatment did not alter the tissue concentration of doxorubicin attained during drug infusion. This result is consistent with the cell culture study<sup>128</sup> and demonstrates that CoQ does not block uptake nor cellular retention of doxorubicin, but it does reduce or prevent the detrimental effects of the drug intracellularly.

Doxorubicin, 0.5 to 3.5  $\mu\text{M}$ , induced a dose-dependent negative inotropic effect in isolated papillary muscle from guinea pig hearts.<sup>6,139</sup> Contractility progressively decreased during a 2.5-hour perfusion with doxorubicin, reflecting the acute cardiotoxic effect of the drug. Partial recovery of contractility was observed when the muscle was perfused without doxorubicin for an additional 3.5 hours. Addition of CoQ to the perfusate after 2.5 hours, together with continued exposure to 3.5  $\mu\text{M}$  doxorubicin, resulted in a gradual return (over 3.5 hours) of contractility to the level before the drug was added. This suggests that the acute cardiotoxic effects of doxorubicin can be reversed and that progressive cardiac damage can be prevented if CoQ is given shortly after the drug is administered. However, the CoQ concentration used in these studies was 100  $\mu\text{g}/\text{mL}$ , a level that is unlikely to be attainable clinically.

In isolated-perfused rabbit hearts, exposure to 8.6  $\mu\text{M}$  doxorubicin causes a progressive decrease of contractility during a 2-hour period.<sup>140</sup> Contractility continued to decrease during a subsequent 3-hour period with a doxorubicin-free perfusate. However, addition of 0.15  $\mu\text{g}/\text{mL}$  CoQ to the doxorubicin-free perfusate resulted in significant, but not complete, recovery of contractility. These results suggest that a very low concentration of CoQ can partially reverse acute anthracycline cardiotoxicity when administered shortly after drug administration.

Administration of CoQ to rabbits, 10 mg/kg/d for 14 days, provided only modest protection from doxorubicin (18  $\mu\text{M}$ )-induced depression of myocardial contractility in hearts isolated from the animals.<sup>8</sup> This suggests that although CoQ may prevent acute doxorubicin-induced cardiotoxicity, administering CoQ only prior to drug exposure does not provide the degree of protection afforded by simultaneous exposure to both.

In rat heart slices, CoQ was ineffective in preventing doxorubicin-induced depression of oxygen consumption.<sup>141</sup> However, although the concentration of CoQ (15  $\mu\text{M}$ ) was substantially higher than normal plasma levels, the concentration of doxorubicin used

(25  $\mu\text{M}$ ) was also much higher than the levels achieved with usual clinical doses.

### **Studies With Laboratory Animals:**

#### **Acute Toxicity**

In mice, doxorubicin given as a single IP dose of 12.5 to 25 mg/kg<sup>43,44,142-147</sup> or two 15 mg/kg<sup>41,42,148</sup> IP doses 4 days apart is highly lethal, with approximately 40% survival after 30 days with the lower doses and 0% to 10% survival after 15 to 30 days with the higher doses. Although the cause of death was not established, administering doxorubicin in these doses is associated with a decrease in body weight,<sup>140,144</sup> a reduction in weight of the heart<sup>42,140</sup> and liver,<sup>42</sup> and a marked increase of plasma and tissue lipid peroxidation.<sup>35,36,41-44</sup> The highest level of tissue lipid peroxides was in the heart, with lower levels in the liver and much lower levels in the kidney and spleen.<sup>35,41</sup> PO doses of 2, 10, and 50 mg/kg CoQ for 10 consecutive days beginning 3 days prior to the first of two 15 mg/kg doses of doxorubicin increased the average life span of mice from 7.5 days to 15 to 16 days with the 2 lower CoQ doses but only to 9.5 days with the highest dose.<sup>41,42,148</sup> However, all animals treated with doxorubicin or doxorubicin plus 50 mg/kg CoQ and 75% of the animals in the other 2 groups died by the end of the 30-day experiment, suggesting that death is only delayed by CoQ. CoQ, 4 intramuscular (IM) doses of 50 mg/kg given 2 and 24 hours before and after 20 mg/kg doxorubicin, reduced lethality from 100% to 50% after 20 days, with 30% of the animals in the CoQ group surviving 30 days.<sup>43,44</sup> In experiments of 26 to 30 days duration, IP CoQ, 10 mg/kg/d for 4 days prior to doxorubicin, improved survival when the drug dose was 15 mg/kg but not at doses of 20 mg/kg and greater.<sup>142-144</sup> Antioxidants other than CoQ, in 15- to 30-day experiments, have also been shown to improve survival of doxorubicin-treated mice, including vitamin E in 4 IM doses (2 before and 2 after 20 mg/kg doxorubicin) of 5, 50, and 500 IU/kg,<sup>43,44</sup> single IP doses of vitamin E of 4100 IU<sup>145</sup> and 3400 IU<sup>146</sup> given 1 day prior to 15 mg/kg doxorubicin, and 1240 mg/kg vitamin C given IP for 7 consecutive days before 15 mg/kg doxorubicin.<sup>147</sup> Similar to the impact of CoQ,<sup>41,42,148</sup> vitamin E appears only to delay the death of mice when the experiment is extended to 60 days.<sup>145</sup> In addition to improving survival, CoQ reduces the impact of doxorubicin on body weight,<sup>140,144</sup> weight of the heart and liver,<sup>42,140</sup> and plasma and tissue lipid peroxides.<sup>41-44</sup> Vitamin E also reduces doxorubicin-induced lipid peroxidation.<sup>35,36,43</sup> Although the results of these studies suggest that CoQ and other antioxidants may reduce anthracycline toxicity, the relevance of these studies to doxorubicin cardiotoxicity in humans is questionable for several reasons, including (1) the



doses of doxorubicin based on body weight (12.5-30 mg/kg) of mice were far higher than doses used clinically (approximately 1.5 mg/kg for a 60-mg/m<sup>2</sup> dose); (2) although the doses of doxorubicin were not excessive on a mg/m<sup>2</sup> basis, that is, a 20-mg/kg dose in mice is 60 mg/m<sup>2</sup>,<sup>37</sup> they are certainly much higher than comparable clinical doses if one considers that a 60-mg/m<sup>2</sup> dose is very lethal to mice but not to humans; (3) since doxorubicin causes severe cellulitis and tissue necrosis when it is injected by other than an IV route (ie, extravasation during attempted IV infusion), lethality following an IP injection may result from causes other than cardiotoxicity; and (4) the doses and routes of administration of antioxidants in most of the studies are unrealistic when considering supplementation for humans. Thus, caution must be exercised in extrapolating the results of the above studies in mice to the clinical setting.

In studies that better reflect toxicity seen clinically, rats given 1 mg/kg/d of IP doxorubicin develop EKG changes (widening of the QRS complex and prolonged QT interval) that are consistent with acute cardiotoxicity.<sup>3-5</sup> Administering 1 mg/kg/d<sup>3</sup> or 1.5 mg/kg/d<sup>5</sup> of IP CoQ beginning 2 days before injection of doxorubicin prevented the development of EKG changes. In addition, when CoQ injections were not started until a total of 12 mg/kg of doxorubicin had been administered, there was restoration of the normal EKG after 7 days.<sup>4</sup> The body weight and heart weight of rats increased during treatment with doxorubicin up to a total dose of 14 mg/kg over 14 days.<sup>5</sup> The body and heart weight gains were similar in rats treated with doxorubicin and with doxorubicin plus CoQ.

The impact of antioxidants other than CoQ on acute doxorubicin toxicity has also been studied in laboratory animals other than mice. In rats, subcutaneous (SC) injection of vitamin A (retinol palmitate), 25 IU/kg/d for 3 days, reduced lethality of a 10-mg/kg IV dose of doxorubicin from 100% to 15% in a 14-day experiment<sup>149</sup> and also reduced doxorubicin-induced lipid peroxidation in the heart. In guinea pigs, doxorubicin is highly toxic, with a 2-mg/kg IP dose being lethal to 100% of the animals within 7 days.<sup>37</sup> Daily IP injections of 835 mg/kg of vitamin C reduced plasma and hepatic lipid peroxides and prolonged survival of the doxorubicin-treated guinea pigs, but all animals still died within 13 days. Doxorubicin is also highly toxic to rabbits, with 100% of animals dying within 7 days of a 7-mg/kg IV dose.<sup>150</sup> Vitamin E, 90 IU/kg/d IM for 14 days before administering doxorubicin, reduced lethality to 34% and also reduced doxorubicin-induced oxidative stress and EKG changes. However, similar to the caution expressed above regarding experiments in mice, the

relevance of these studies to acute anthracycline cardiotoxicity in humans is questionable.

### **Studies With Laboratory Animals:**

#### **Chronic Toxicity**

Rabbits given IV doxorubicin, 1 mg/kg 3 times weekly every other week for 4 months (maximum dose: 25 mg/kg = 450 mg/m<sup>2</sup>), develop severe histological changes in the heart that are characteristic of doxorubicin-induced cardiomyopathy.<sup>22,151</sup> The rabbits also exhibit marked EKG changes and elevation of the creatine phosphokinase level. Although less toxic than a single 7-mg/kg IV dose of doxorubicin,<sup>150</sup> the drug was still lethal to rabbits when administered in this regimen, with 3 of 4 rabbits dying after cumulative doses of 12, 13, and 19 mg/kg and only 1 animal surviving a cumulative dose of 25 mg/kg. Body weight increased in all rabbits throughout the experiment, except for a decline in weight after a total dose of 20 mg/kg in the animal that survived a dose of 25 mg/kg. When IV CoQ, 2.5 mg/kg, was administered with each dose of doxorubicin to another group of 4 rabbits, 2 died after cumulative doses of 23 and 24 mg/kg doxorubicin and 2 survived a cumulative dose of 25 mg/kg. Animals in the CoQ group exhibited only very minimal histological changes in the heart, minimal EKG changes, and a gain in body weight throughout the experiment, suggesting that CoQ prevents the development of doxorubicin-induced cardiomyopathy. In another study by the same authors,<sup>21</sup> 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.

Giving rabbits IV doxorubicin, 0.8 mg/kg on 3 consecutive days each week for 3 months, also results in histopathological changes in the heart and EKG changes (flattened/inverted T waves and decreased QRS voltage) that are characteristic of doxorubicin-induced cardiomyopathy.<sup>140</sup> CoQ, IV doses of 0.1 or 0.4 mg/kg 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 doxorubicin (2 mg/kg IP once weekly for 18 weeks) in rats also results in

histological changes in the heart that are characteristic of doxorubicin-induced cardiomyopathy.<sup>41</sup> As in rabbits, administering CoQ (10 mg/kg IM 6 days per week) prevents the development of cardiomyopathic changes.

The impact of acute versus chronic administration of doxorubicin on damage to cardiac mtDNA was investigated in mice given IP doxorubicin, 4 mg/kg/d for 6 consecutive days (acute toxicity), 1 mg/kg weekly for 12 weeks (chronic toxicity), or 2 mg/kg for 12 weeks (chronic toxicity).<sup>40</sup> Additional groups of animals receiving 1 and 2 mg/kg/wk of doxorubicin also received PO CoQ, 5 or 10 mg/kg/d. A substantial mtDNA deletion was detected in both groups of animals treated with doxorubicin for 12 weeks. The incidence of the lesion was 33% in the low-dose doxorubicin group and 80% in the high-dose doxorubicin group. In the 1-mg/kg/wk doxorubicin group, the incidence of the mtDNA lesion was reduced to 7% by 5 mg/kg/d of CoQ and to 0% by 10 mg/kg/d of CoQ. In the 2-mg/kg/wk doxorubicin group, the incidence of the mtDNA lesion was reduced to 40% by 5 mg/kg/d of CoQ and to 30% by 10 mg/kg/d of CoQ. Both doses of CoQ also significantly reduced doxorubicin-induced oxidative stress in cardiac mitochondria. In contrast to the mice treated for 12 weeks, mice treated with doxorubicin for only 6 days did not exhibit the mtDNA lesion. These results suggest that doxorubicin-induced mtDNA damage, which is reduced by administration of CoQ, does not contribute to the drug's acute cardiotoxicity but that it may be a factor in the development of doxorubicin-induced cardiomyopathy.

The short-term and long-term impact of doxorubicin on the ETS of heart and liver mitochondria were investigated in rats treated with a total of 15 mg/kg doxorubicin in 6 divided IP doses over 2 weeks.<sup>152</sup> Animals in another group received doxorubicin plus a 0.2% CoQ diet. Four and 28 weeks following the last dose of doxorubicin, mitochondria from the heart and liver were isolated and complex I activity was determined. In cardiac mitochondria, complex I activity was unchanged 4 weeks after doxorubicin treatment but significantly reduced at 28 weeks. No significant decrease in complex I activity was seen at 4 or 28 weeks in animals fed the CoQ-containing diet. Doxorubicin treatment did not affect complex I activity of liver mitochondria. These results demonstrate that the impact of doxorubicin on mitochondria is cardioselective; that it is a delayed phenomenon, possibly reflecting an impairment of the regenerative capacity of mitochondria; and that CoQ prevents doxorubicin-induced mitochondrial damage.

The impact of antioxidants other than CoQ on the development of chronic doxorubicin-induced cardiotoxicity in rabbits has been reported by several investigators.<sup>23,24,34,153</sup> In the control groups (no antioxidants), rabbits receiving IV doxorubicin at a dose of 1.2 to 2.4 mg/kg/wk for a period of 9 to 17 weeks developed histopathological changes in the heart that are characteristic of doxorubicin-induced cardiomyopathy. The antioxidant and antioxidant combinations investigated include vitamin E (13 IU/kg) plus sodium selenite (0.03 mg/kg) given IM 24 hours prior to each doxorubicin injection,<sup>23</sup> 450 IU/kg vitamin E given IP 5 times/wk,<sup>24</sup> daily PO administration of 30 IU/kg vitamin E plus 125 IU/kg vitamin A,<sup>34</sup> and 25 IU/kg vitamin E, 25 IU/kg vitamin E plus 0.06 mg/kg sodium selenite, or 250 IU/kg vitamin E given IM 24 hours prior to each doxorubicin injection.<sup>153</sup> The antioxidant/antioxidant combinations were shown to prolong survival of animals by 25% to 40% in 1 experiment,<sup>153</sup> but survival was not prolonged in 2 others.<sup>23,24</sup> The vitamin E/vitamin A combination reduced cardiac oxidative stress but did not prevent EKG changes induced by doxorubicin.<sup>34</sup> Although a modest reduction in the doxorubicin-induced histopathological changes in the heart were reported with some of the antioxidant treatments,<sup>23,34,153</sup> vitamin E in the highest dose used did not provide any protection.<sup>24</sup> In dogs, 25 IU/kg IM vitamin E or 25 IU/kg vitamin E plus 0.06 mg/kg sodium selenite, given weekly on the same day that a 1-mg/kg IV dose of doxorubicin was administered, did not improve survival nor alter the development of cardiomyopathy.<sup>154</sup> Thus, in contrast to the studies that demonstrate prevention of doxorubicin-induced cardiomyopathy by CoQ, these studies suggest that antioxidant protection alone provides, at best, only minimal protection from the chronic toxicity of anthracyclines.

### Clinical Studies

Judy et al<sup>155</sup> investigated the impact of CoQ on the development of doxorubicin-induced cardiotoxicity in lung cancer patients with normal and low cardiac function. Fourteen adult patients with normal resting cardiac function received 50 to 70 mg/m<sup>2</sup> of doxorubicin at regular intervals (n = 7) or doxorubicin plus 100 mg/d of PO CoQ beginning 3 to 5 days before the first dose of doxorubicin and continuing until therapy was completed (n = 7). After a total cumulative dose of 600 mg/m<sup>2</sup> doxorubicin, patients not taking CoQ 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. After a 600-mg/m<sup>2</sup> cumulative dose of doxorubicin in patients receiving CoQ, cardiac function remained unchanged from that mea-

sured before therapy was started. In addition, the 7 patients taking CoQ continued to receive doxorubicin until a total cumulative dose of 900 mg/m<sup>2</sup> was administered, a dose at which approximately 50% of patients treated with doxorubicin can be expected to develop CHF.<sup>10</sup> Following administration of 900 mg/m<sup>2</sup> of doxorubicin to 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. Patients (2 groups of 4 each) with low resting cardiac function prior to doxorubicin therapy were treated similarly to those who entered the study with normal cardiac function. Patients with low cardiac function before therapy tolerated doxorubicin poorly, exhibiting a profound reduction from their baseline cardiac function following a total cumulative dose of 420 mg/m<sup>2</sup>. Patients taking CoQ received 580 mg/m<sup>2</sup> of doxorubicin but exhibited a lesser decrement of cardiac function than did those who received 420 mg/m<sup>2</sup> of the drug without CoQ. The results of this study suggest that CoQ prevents doxorubicin-induced cardiomyopathy and that it may be possible to escalate the total cumulative dose of doxorubicin when CoQ is administered concurrently with the drug. However, since cardiac performance was not measured beyond the end of therapy, the impact of CoQ on cardiomyopathy that is not manifest until months or years after completion of therapy was not determined. It is also not possible to determine or estimate the efficiency of CoQ absorption in this study since CoQ blood levels were not measured and the CoQ formulation and time of CoQ administration relative to meals was not stated.

Cortes et al<sup>156,157</sup> measured the systolic time interval (STI; pre-ejection period/left ventricular ejection time) in 18 adult patients treated with 50 mg/m<sup>2</sup> doxorubicin (total cumulative dose of 200-500 mg/m<sup>2</sup>), 1.4 mg/m<sup>2</sup> vincristine, and 500 mg/m<sup>2</sup> cyclophosphamide every 4 weeks. Eight of 10 patients receiving chemotherapy alone exhibited a progressive prolongation of STI (reflecting depressed left ventricular function) with increasing cumulative doses of doxorubicin, and 2 patients developed CHF after 200 and 350 mg/m<sup>2</sup> of doxorubicin. In only 2 of 8 patients receiving chemotherapy plus 50 mg/d of PO CoQ, an increase in STI was detected, although 1 patient did develop CHF after 350 mg/m<sup>2</sup> of doxorubicin. Although these investigators used only a small dose of CoQ and the formulation and time of administration relative to a meal were not stated, the results suggest that CoQ may prevent the development of doxorubicin-induced cardiotoxicity.

Iarussi et al<sup>158</sup> measured cardiac function in children with hematological malignancies who were

treated with equal amounts of doxorubicin and daunorubicin (mean cumulative combined dose: 240 mg/m<sup>2</sup>) or the anthracyclines (mean cumulative combined dose: 252 mg/m<sup>2</sup>) plus CoQ, 100 mg PO twice daily (formulation and time of administration relative to meals not stated) for the duration of the study. Echocardiographic evaluation of ventricular function was done before therapy started, after a total anthracycline dose of 180 mg/m<sup>2</sup>, and at the completion of therapy. Left ventricular fractional shortening was reduced in both groups, although it occurred later and to a lesser degree in patients receiving CoQ. Only patients in the group not receiving CoQ exhibited depressed interventricular septal wall thickening. Although the impact of chemotherapy at an extended time interval following treatment was not done, these results suggest that CoQ reduces anthracycline-induced cardiotoxicity.

Folkers et al<sup>159,160</sup> measured cardiac output in 6 adult patients with adenocarcinoma of the lung who were treated every 3 to 4 weeks with doxorubicin (3-5 infusions, total cumulative dose: 250-361 mg), 4 patients receiving 3 to 4 infusions of doxorubicin (total cumulative dose: 215-355 mg) plus 60 mg/d PO CoQ (formulation and time of administration relative to meals not stated), and 5 patients receiving 2 infusions of doxorubicin (total cumulative dose: 145-175 mg) plus 60 mg/d PO CoQ. All patients receiving doxorubicin without CoQ exhibited a 25% to 40% reduction in cardiac output (compared to that before treatment started) following the second (3 patients) or third (3 patients) drug infusion. In patients receiving CoQ, 1 exhibited a 16% reduction of cardiac output following the fourth doxorubicin infusion, 1 exhibited an 18% reduction of cardiac output following the third infusion, and 1 had a transient reduction of cardiac output following the second infusion, but after the third and fourth infusions, cardiac output was not significantly different from that measured before treatment started. These studies further suggest that doxorubicin cardiotoxicity may be prevented by CoQ, but as in the above studies, the long-term effect of doxorubicin was not determined.

Okuma and Ota<sup>161</sup> randomized 80 patients with various types of malignancies to receive doxorubicin or doxorubicin plus CoQ 90 mg/d PO beginning 1 week before chemotherapy was started and continuing until treatment was completed. Patients received 3 to 10 infusions with a total cumulative doxorubicin dose of 118 to 517 mg (doxorubicin-only group) or 123 to 517 mg (doxorubicin plus CoQ). Patients in the doxorubicin-only group 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, suggesting that CoQ protects patients from the cardiotoxicity of doxorubicin.

Several other studies also suggest that CoQ prevents the EKG changes that occur during therapy with doxorubicin. Takimoto et al<sup>162</sup> investigated the impact of CoQ, 90 mg/d PO, in a randomized study of 40 patients with lung, breast, and thyroid cancer who were treated with doxorubicin (50 mg/m<sup>2</sup>), cyclophosphamide, and 5-fluorouracil plus radiation therapy. They reported 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<sup>163</sup> reported that IV infusion of 1 mg/kg/d of CoQ for 4 days beginning 1 day before chemotherapy, reduced EKG changes induced by doxorubicin or daunorubicin. Yamamura<sup>164</sup> reported a similar effect of CoQ, 30 mg/d PO, in patients being treated with doxorubicin.

Legha et al<sup>165</sup> investigated the impact of vitamin E on the development of doxorubicin-induced cardiomyopathy in 21 women with breast cancer who were being treated with doxorubicin, cyclophosphamide, and 5-fluorouracil. Vitamin E, 3000 IU/m<sup>2</sup>/d PO, beginning 7 days before and continuing throughout chemotherapy, resulted in a 6- to 8-fold increase in the blood level of the antioxidant. Three of the 21 women developed CHF at total cumulative doses of 470, 550, and 820 mg/m<sup>2</sup> doxorubicin. Cardiac biopsies were performed in 12 patients after total doxorubicin doses of 450 to 550 mg/m<sup>2</sup> (14 biopsies) and 560 to 660 mg/m<sup>2</sup> (5 biopsies). Histopathological grading of the biopsies revealed no difference from biopsies of patients treated with comparable doses of doxorubicin but not vitamin E. Weitzman et al<sup>166</sup> randomized 16 adult patients to receive chemotherapy with or without 1800 IU/d of PO vitamin E. The mean cumulative dose of doxorubicin in the control and vitamin E groups was 260 (range, 120-453) and 247 (range, 53-353) mg/m<sup>2</sup>, respectively. A progressive decline of the STI was seen in all patients, with no significant difference between the 2 groups. The results of these studies suggest that vitamin E offers no protection from the development of doxorubicin-induced cardiomyopathy.

## CoQ/Antioxidants and Anthracycline Antineoplastic Activity

### Mechanism of Action of Anthracyclines

Several mechanisms have been proposed to account for the antineoplastic activity of anthracyclines, including inhibition of topoisomerase II, free radical

generation, and membrane-related actions. Controversy surrounds the subject, mostly related to the role, if any, that free radicals and lipid peroxidation play in the antitumor activity of these drugs. Although anthracyclines generate high levels of oxidative stress, several lines of evidence suggest that this activity does not contribute to the anticancer activity of these drugs. Evidence against a free radical mechanism of action include the following: (1) most evidence that favors a free radical-dependent model, as has been thoroughly reviewed by others,<sup>49,167,168</sup> has been obtained using in vitro systems and anthracycline concentrations that far exceed those that are achieved clinically; (2) as discussed above, doxorubicin induces far lower levels of oxidative stress in cancer cells than in normal cells<sup>130</sup> because of lower levels of free radical-generating systems and higher levels of antioxidants<sup>131-138</sup>; (3) anthracyclines are located primarily in the nucleus of drug-sensitive cancer cells,<sup>115-119</sup> 99.7% of intranuclear doxorubicin is bound to DNA,<sup>120</sup> and doxorubicin intercalated into DNA cannot be reduced<sup>169</sup>; (4) at usual clinical concentrations of doxorubicin (up to 2.8 μM), drug uptake, drug metabolism, and the frequency of DNA-protein-associated single-strand breaks and DNA-protein cross-links are the same in hypoxic and euoxic cancer cells<sup>170</sup>; (5) doxorubicin-sensitive and doxorubicin-resistant cancer cells generate comparable levels of free radicals in the presence of the drug<sup>171</sup>; (6) antioxidant depletion does not enhance the cytotoxicity of doxorubicin<sup>167</sup>; and (7) levels of antioxidants and antioxidant enzymes are not elevated in anthracycline-resistant cells.<sup>172</sup> In contrast to evidence against a free radical mechanism of action, a substantial body of evidence exists that supports topoisomerase II inhibition as the primary mechanism accounting for the antineoplastic activity of anthracyclines,<sup>49,167,168</sup> including (1) topoisomerase II-mediated DNA damage by anthracyclines occurs at clinically relevant drug concentrations, (2) cytotoxicity of anthracyclines correlates with topoisomerase II-mediated DNA damage, and (3) altered topoisomerase II activity (cellular levels, structure, and function) confers resistance to anthracyclines as well as to other topoisomerase II inhibitors such as the epipodophyllotoxins (eg, etoposide). Further evidence against a free radical mechanism of action is in the results of preclinical and clinical studies showing that antioxidants do not interfere with the cytotoxicity of anthracyclines.

### Studies With Cell Cultures

The effect of CoQ on the antineoplastic activity of doxorubicin has been studied in murine L-1210 leukemia cells<sup>151</sup> and Ehrlich ascites carcinoma cells.<sup>130</sup> L-1210 cells in logarithmic growth phase are very sensi-

tive to doxorubicin, with a drug concentration of 0.17  $\mu\text{M}$  being cytotoxic to all cells within 5 days and a doxorubicin concentration of 0.017  $\mu\text{M}$  inhibiting cell growth by 40% after 5 days. The addition of 46  $\mu\text{M}$  CoQ increases the cytotoxic activity of doxorubicin, with 100% cell death in 3 days with the higher drug concentration and complete inhibition of cell growth (viable cells remained unchanged from the start of the experiment) after 5 days of exposure to the lower drug concentration. Exposure of Ehrlich ascites cells to 34.5  $\mu\text{M}$  doxorubicin for 45 hours resulted in a 95% inhibition of DNA synthesis, an effect that was not altered by the addition of an equimolar concentration of CoQ.

Vitamin E, at a concentration similar to that of human plasma, has been shown to increase the cytotoxic effects of doxorubicin on mouse neuroblastoma cells and rat glioma cells in culture.<sup>173</sup> In cultured human colorectal cancer cells, vitamin E, at approximately 100 times the usual human plasma level, reduced the doxorubicin concentration for 50% inhibition of cell growth by 60% and 90% in 2 different cell lines.<sup>174</sup> Vitamin C, 1  $\mu\text{M}$ , increased the cytotoxicity of doxorubicin in 2 human breast cancer cell lines,<sup>175</sup> and vitamin A, 2.5 to 15  $\mu\text{M}$ , enhanced doxorubicin cytotoxicity in human erythroleukemia cells in culture.<sup>176</sup>

### Studies With Laboratory Animals

Shaeffer et al<sup>144</sup> investigated the impact of CoQ on the antineoplastic activity of doxorubicin in mice injected IV with Dunn osteosarcoma cells. Mice received IP injections of CoQ, 10 mg/kg/d, for 4 consecutive days. Tumor cells suspensions were injected on the fourth day, and 1 day later, the mice were treated with 10 mg/kg IP or 15 mg/kg IV doxorubicin. Control mice received doxorubicin without the CoQ pretreatment. After 21 days, mice were sacrificed and lung tumors were counted. Compared to mice that received doxorubicin alone, mice treated with CoQ and doxorubicin developed 25% to 30% fewer pulmonary tumors. Others have demonstrated that 5 mg/kg CoQ plus 1 mg/kg doxorubicin IP every other day, compared to administration of doxorubicin alone, increases the duration of survival of mice injected with L-1210 leukemia cells by 70%.<sup>44</sup>

Doxorubicin, 4 mg/kg/wk IP for 4 weeks, significantly prolonged survival from a mean of 15 days (controls not receiving doxorubicin) to 30 days in Ehrlich ascites carcinoma-bearing mice and increased the percentage of long-term (60-day) survivors from 0% to 30%.<sup>177</sup> Mice treated with vitamin E, 250 IU/kg/d PO, in addition to doxorubicin had a mean survival of 36 days, and 50% were long-term survivors. Vitamin E, 50 IU/kg IP every other day, modestly prolonged the

survival of L-1210 leukemia-bearing mice that were treated with IP doxorubicin, 1 mg/kg every other day.<sup>44</sup> Myers et al<sup>36</sup> also reported that vitamin E (1 IP dose of 3400 IU/kg) prolonged survival of P-388 ascites tumor-bearing mice treated with a single IP dose of 7.5, 10, 12.5, or 15 mg/kg doxorubicin. Although mice in all groups survived longer than untreated tumor-bearing mice, the mean survival time of doxorubicin-treated mice was inversely related to the drug dose, illustrating the high lethality of the drug in mice. Vitamin E, 3200 IU/kg, given SC 1 day before IV doxorubicin (7.5 mg/kg on day 1 and 10 mg/kg on day 15) did not affect survival of rats bearing acute myeloid leukemia,<sup>178</sup> although a single vitamin E dose of 140 IU/kg markedly enhanced the anticancer effect (tumor volume, metastasis, and tumor regression) of doxorubicin (5 mg/kg/wk times 3) in rats with transplanted prostate adenocarcinoma.<sup>179</sup> Daily IP injections of 2 g/kg of vitamin C did not alter the survival of L-1210-bearing mice treated with a single IP dose of 5 mg/kg doxorubicin,<sup>37</sup> although 2 IP injections of 1 g/kg vitamin C given 24 and 3 hours before a single IP dose of doxorubicin in mice with neoplastic ascites cells increased the life span by 70% compared to mice treated with doxorubicin alone.<sup>180</sup> Four daily doses of vitamin A, 167 mg/kg/d IP,<sup>181</sup> and a single dose of  $\beta$ -carotene, 10 mg/kg IP,<sup>182</sup> also prolonged survival of tumor-bearing mice treated with IP doxorubicin, 0.2 mg/kg/d times 4 or 1.75 mg/kg/d times 4, respectively. Although many of the antioxidants in these studies were administered in doses that far exceed reasonable clinical doses, the data suggest that antioxidants do not interfere with the antineoplastic activity of doxorubicin.

Myelosuppression is also a reflection of the cytotoxicity of antineoplastic agents. In rabbits, CoQ, 2.5 mg/kg/d IV 3 times weekly,<sup>22</sup> and vitamin E, 270 IU/kg/d IM for 14 days,<sup>150</sup> 450 IU/kg/d IP 5 times per week,<sup>24</sup> and 12 IU/kg IM before each IV doxorubicin injection,<sup>23</sup> did not alter the pancytopenia that develops during treatment with doxorubicin, although a single IP dose of 3400 IU/kg vitamin E administered 24 hours before doxorubicin markedly enhanced the pancytopenia caused by the drug.<sup>183</sup> Thus, these antioxidants do not appear to prevent the cytotoxicity of doxorubicin to rapidly proliferating bone marrow cells, although a very high dose of vitamin E may enhance it.

### Clinical Studies

Antitumor activity and the degree of myelosuppression was unaffected by PO vitamin E, 3000 IU/m<sup>2</sup>/d, in women with breast cancer who were treated with doxorubicin (total cumulative dose of

100-820 mg/m<sup>2</sup>), 5-fluorouracil, and cyclophosphamide,<sup>165</sup> or by PO CoQ, 50 mg/d, in patients with advanced cancer who were treated with doxorubicin (50 mg/m<sup>2</sup>), vincristine, and cyclophosphamide every 4 weeks.<sup>156</sup> Alopecia, resulting from doxorubicin cytotoxicity to rapidly proliferating hair follicle cells, is also unaffected by CoQ in daily PO doses of 50 mg/d<sup>165</sup> or 120 mg/d<sup>184</sup> or by 1 mg/kg/d IV CoQ beginning 1 day before and continuing for 2 days after treatment with doxorubicin or daunorubicin.<sup>163</sup> These studies suggest that CoQ and vitamin E do not interfere with the cytotoxic effects of anthracyclines.

### **Antioxidants and Antineoplastic Activity**

Prevailing evidence suggests that anthracyclines exert their antineoplastic activity by their interaction with DNA, most likely by inhibition of topoisomerase II activity. The evidence also supports the contention that oxidative stress induced by the drugs does not play a role in their antitumor effects. The experiments cited above in this section provide additional evidence that oxidative stress is not responsible for the drugs' antineoplastic activity and that counteracting drug-induced oxidative stress with antioxidants may even enhance the cytotoxicity of anthracyclines. Enhancement of cytotoxicity by antioxidants may be due to the impact of oxidative stress on cell proliferation and drug-induced apoptosis.

Oxidative stress reduces cell growth by inhibiting progression through the cell cycle, causing cells to remain in the G1 (pre-DNA synthesis phase) or to become quiescent (G0 phase).<sup>33,185-190</sup> Thus, the rate of cancer cell proliferation in culture<sup>131,191-193</sup> and the growth of tumors in laboratory animals<sup>194-196</sup> decrease during periods of oxidative stress, and rapid rates of tumor growth are associated with low levels of oxidative stress.<sup>185,197</sup> The effects of oxidative stress are most likely due to various aldehydes, the secondary products of lipid peroxidation,<sup>33</sup> which have been shown to mediate growth inhibition in cell cultures and in animals.<sup>198,199</sup> Anthracyclines require cells to progress through their cell cycle for the drugs to exert their cytotoxic activity. This is because the activity of topoisomerase II, which releases torsional strain of double-stranded DNA during periods of replication and transcription, is necessary only when cells are progressing through their cell cycle. The content of topoisomerase II is far higher in rapidly proliferating cancer cells than in quiescent cancer cells, and cancer cells have been shown to be sensitive to the cytotoxic effects of topoisomerase II inhibitors only during periods of rapid growth.<sup>200,201</sup> In addition to effects on cell cycle progression, oxidative stress interferes with

drug-induced apoptosis,<sup>202,203</sup> most likely by inhibition of caspases (enzymes that carry out cell disassembly<sup>204-206</sup>) and possibly by inactivating death receptors.<sup>207</sup> Thus, administering CoQ and other antioxidants to counteract anthracycline-induced oxidative stress may enhance the antineoplastic effects of these drugs.

### **Summary and Discussion**

Anthracyclines induce an acute but reversible depression of myocardial function and a chronic irreversible cardiomyopathy, both of which appear to be preventable by administering CoQ concurrently with anthracyclines. The unique structure of cardiac mitochondria, which possess an NADH dehydrogenase on the outer (cytosolic) surface of the inner mitochondrial membrane, most likely explains why the drugs are selectively toxic to the heart. Reduction of the anthracycline to its semiquinone by this enzyme results in an extremely high level of oxidative stress in cardiac mitochondria. Two mechanisms explain the generation of oxidative stress: (1) the reduced anthracycline transfers an electron to molecular oxygen resulting in formation of superoxide radicals and (2) the anthracycline aglycones, which form after reduction of the parent drug, penetrate the inner membrane, displace CoQ from the ETS, and transfer electrons received from complex I and complex II to molecular oxygen. In addition, displacement of CoQ by the aglycones disrupts mitochondrial energetics, which may account for the acute cardiotoxic effects of the anthracyclines. The ultimate damage to mitochondria, however, appears to be oxidative damage to mtDNA, which interferes with the regenerative capacity of the organelle. Once this irreversible damage occurs, the myocytes are destined to undergo apoptosis or necrosis, an event that may not occur until months or years after chemotherapy is completed.

Several mechanisms may explain the cardioprotective effect of CoQ during chemotherapy with anthracyclines. CoQ may prevent the anthracycline from being reduced to its semiquinone by the cytosolic NADH dehydrogenase, possibly by competition for the enzyme active site. If this were the mechanism, CoQ would be reduced to its hydroquinone instead of the anthracycline being reduced to its semiquinone. CoQ may undergo an oxidation-reduction reaction, with the anthracycline semiquinone resulting in formation of CoQ hydroquinone and the unreduced anthracycline. Either of these mechanisms would prevent superoxide generation by the reduced anthracycline within the mitochondrial cytosol and prevent formation of the anthracycline aglycones, which generate superoxide radicals from the ETS. Alternatively, CoQ

may compete with the aglycones for the CoQ site in the ETS, or CoQ may simply be acting as an antioxidant to reduce oxidative stress within cardiac mitochondria. However, since antioxidants other than CoQ do not prevent the development of anthracycline-induced cardiomyopathy, CoQ acting only as an antioxidant seems unlikely.

Although the use of CoQ for prevention of anthracycline-induced cardiotoxicity appears promising, many unanswered questions remain, and further clinical research is certainly warranted. For example, if CoQ prevents the development of anthracycline-induced cardiomyopathy, as it appears to, is the protection long standing? Thus, long-term follow-up of patients receiving CoQ during anthracycline-based chemotherapy is necessary to determine if the supplement prevents the development of cardiomyopathy that may not develop until years after therapy is completed. Much larger randomized controlled trials need to be conducted, and the optimal CoQ dosage needs to be determined. Future studies need to use a CoQ regimen that optimizes CoQ absorption (ie, using a highly absorbable formulation and administering CoQ with a meal), and CoQ blood levels need to be measured so that CoQ absorption can be documented. Does CoQ influence the pharmacokinetics of anthracyclines (ie, Does CoQ increase, decrease, or have no effect on the rate of metabolism or excretion of the parent pharmacologically active anthracycline)? and does CoQ affect the proportion of the parent drug metabolized to pharmacologically active metabolites (eg, doxorubicinol) versus inactive metabolites (the aglycones)? CoQ may affect pharmacokinetics in a manner that enhances the antineoplastic activity of the drugs, as suggested by studies cited above, although enhancing the cytotoxicity of anthracyclines may also enhance side effects that result from the drugs' cytotoxicity to rapidly proliferating normal cells (eg, myelosuppression). Certainly, the impact of CoQ on the antitumor activity of anthracyclines needs to be determined in short-term and long-term studies. If it can be established that CoQ prevents anthracycline-induced cardiotoxicity while preserving (or enhancing) antineoplastic activity, the use of higher drug dosages with resulting improvement in the clinical response may be possible.

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