Doxorubicin-Induced Dilated Cardiomyopathy: Phisiopathological Mechanisms and Therapeutic Alternatives.

Yanet Hernández-Matos[1], Livan Delgado Roche[1]

1. Center of Studies for Research and Biological Evaluations (Departament of Biochemistry). Pharmacy and Food Sciences College, University of Havana. Havana, Cuba.

*Author for correspondence: Ave. 23 # 21425 e/ 214 y 222, La Coronela, La Lisa. Ciudad de la Habana. Cuba. E-mail: ymatos@ifal.uh.cu

Summary

The doxorubicin (DOX) is one of the most effective chemotherapeutic agents against a wide variety of cancers. However, its use is seriously limited by the development in the heart of acute and chronic toxic effects. Oxidative stress is the major etiopathological factor in DOX-induced cardiotoxicity. Mechanisms of action and toxicity of DOX are revised in this review, an also the potential therapeutic alternatives. The mechanism of doxorubicin-induced dilated cardiomyopathy is characterized by left ventricular dilation that is associated with systolic dysfunction also the strategies to attenuate DOX toxicity are dosage optimisation, synthesis and use of analogues or combined therapy with antioxidants. The most promising results come from the combination of the drug delivery together with an antioxidant in order to reduce oxidative stress.

Key Words: doxorubicin, dilated cardiomyopathy, oxidative stress, antioxidant defense.

Introduction

The doxorubicin (DOX) is an excellent drug for the treatment of a wide variety of human tumors. However, the development of irreversible cardiotoxicity has limited its use. Due to the successful action of DOX as a chemotherapic agent, several strategies have been tried to prevent/attenuate the side effects of DOX (1). DOX-induced cardiotoxicity is thought to be a complex multifactorial process, which includes oxidative stress. DOX localizes to the mitochondria and is highly susceptible to enzymatic reduction to generate superoxide radicals and reactive oxygen species (ROS), which can cause mitochondrial swelling and ultrastructural changes and alter mitochondrial function (2). ROS play an important role in the pathogenesis of the chronic cardiotoxicity and are in part generated by redox-cycling of DOX. DOX also enters mitochondria where it either directly inhibits the respiratory chain by binding to cardiolipin, or interacts with mitochondrial DNA (mtDNA). Somatically acquired and accumulating respiratory chain defects which perpetuate ROS formation and contributing quantitative and qualitative lesions in mtDNA (mtDNA depletion, deletions, and point mutations) represent a plausible mechanism for the delayed onset of the cardiomyopathy and the molecular basis of the dose memory for the cumulative doxorubicin exposure (3).

DOX-induced oxidative stress in cardiomyocyte mitochondria is associated with cell death and responsible for cardiac damage (2). DOX increases cardiomyocyte susceptibility by reducing SOD activity and therefore reducing the ability of cardiac cells to inactivate ROS. Oxidative stress also has been shown to cause depolarization of the mitochondrial membrane, leading to programmed cell death (4). The production of free radicals and oxidative stress is closely involved with DOX action, regarding both anti-tumour and toxic effects (5).

Myocardial mitochondria also play an important role in the physiological regulation of the intracellular homeostasis of calcium ions by either sequestering or releasing Ca2+. It is not known whether the alterations in myocellular Ca2+ homeostasis are primary or secondary to the respiratory chain dysfunction in chronic DOX cardiomyopathy. In vitro models suggest that the DOX-induced Ca2+ uptake by cardiomyocytes and their mitochondria inhibits Complexes I and IV of the respiratory chain and enhances ROS generation, effects which may subsequently limit ATP production. These data are supported by the observation that decreased cellular ATP levels and thus mitochondrial dysfunctions were preceded by increases in mitochondrial Ca2+ (3).

DOX-induced cardiomyophaty: Dilated cardiomyophaty

DCM, the most common of the cardiomyopathies, is characterized by an increase in both myocardial mass and volume. The walls become thin and stretched, compromising cardiac contractility and ultimately resulting in poor left ventricular function. DCM may occur at any age, but is common in males between the ages of 20 and 50 years (6). Genetically inherited (familial) forms of DCM have been identified in 25–35% of patients presenting with this disease, but many other acquired conditions may result in an identical clinical presentation and pathological function. These conditions include alcohol-induced cardiomyopathy, peripartum cardiomyopathy, haemochromatosis, chronic anaemia, non-compaction cardiomyopathy, DOX toxicity, sarcoidosis and viral myocarditis. DCM may also occur secondary to ischaemic heart disease, valvular heart disease, hypertension and congenital heart disease (7). In cases where an underlying pathology cannot be identified, the patient is diagnosed with an idiopathic dilated cardiomyopathy (iDCM). The 5 year survival after diagnosis is 50%, as patients often develop progressive congestive heart failure (CHF) and complications such as thromboembolic conditions and arrhythmias. Mutations in lamin A/C are often associated with conduction system disease; desmin and dystrophin with a skeletal myopathy; desmoplakin and plakoglobin are often associated with wooly hair and keratoderma. Mutations in d-sarcoglycan have been found to cause loss of skeletal muscle and a form of muscular dystrophy (8).

DOX is very effective anticancer drug that is prescribed worldwide. However, many patients treated with this drug, irrespective of age, develop insidious DCM and heart failure. The scope of the problem remains to be adequately defined. Causative mechanism of the disease is multifactorial, but seems to be associated with generation of ROS, disruption of mitochondria, and uncoupling of the electron-transport chain (9, 10). Use of dexrazoxane might be cardioprotective by attenuation of the formation of free radicals. Early detection of disease by both non-invasive and serological techniques shows promise (11).

Left ventricular non-compaction

Left ventricular non-compaction can be difficult to diagnose unless the physician has a high level of suspicion during echocardiographic assessment. With careful review of echocardiograms and other clinical data, this disorder seems to be common in children, and is also reported in adults (12). About 9% of all cases of cardiomyopathy are diagnosed as left ventricular noncompaction, with only DCM and hypertrophic cardiomyopathy being more common. In the most recent American College of Cardiology/American Heart Association (AHA/ACC) cardiomyopathy classification, left ventricular non-compaction was recognised for the first time as a formal form of cardiomyopathy (13). A substantial percentage of these patients have a dilated left ventricle with systolic dysfunction, mimicking DCM. Signs, symptoms, and outcomes of these patients mirror those of patients with pure DCM, but in young children, outcomes are worse than for those with DCM.

Doxorrubicin: molecular mechanisms and toxicity

Adriamycin, also called DOX, is an antibiotic anthracycline that was isolated from a pigment of *Streptomyces peacetius* in the early 1960s but which is now chemically synthesised. Having been employed for more than 30 years in the battle against cancer, DOX is essential in treating breast and oesophageal carcinomas, solid tumours in childhood, osteosarcomas, Kaposi's sarcoma, soft tissue sarcomas, and Hodgkin and non-Hodgkin lymphomas (14, 15). Anthracyclines (doxorubicin, daunorubicin, epirubicin, and idarubicin) are the most effective anti-neoplastic family in current clinical practice.

Specifically, DOX is a key chemotherapeutic drug for cancer treatment; although its use is limited by the chronic and acute toxic side effects it produces (15). Acute side effects related to intravenous injection of DOX appear within minutes after infusion, including nausea, vomiting, myelosuppression and arrhythmia. On the other hand, chronic effects may develop several weeks or even months after the recurrent administration of the drug, provoking heart, liver, brain or kidney injury. Since cardiomyocytes, as well as neurons, are post-mitotic cells, the vast majority of the damage is irreversible, and unalterably affects cardiac and brain functions. Furthermore, heart repercussions are even more prevalent because of this organ's greater sensitivity to damage induced by free radicals, given the high oxidative metabolism of the heart and its lower level of antioxidant defences.

DOX-related cardiotoxicity may cause dose-dependent cardiomyopathy and congestive heart failure; moreover, persistent changes of cognitive function (memory and concentration loss and difficulty of performing multiple tasks) may occur (15, 16). Dosage-dependent chronic cardiomyopathy associated with DOX administration generates marked hypotension, tachycardia, cardiac dilatation and ventricular failure. At the serum level, increased glutamate oxalacetic transaminase, lactate dehydrogenase, and creatinine phosphokinase enzyme activities have been noted.

At the ultrastructural level, myofibril loss, mitochondrial swelling, cytoplasmatic vacuolization, and an increased number of lysosomes have been reported (17, 18). Toxicity associated with DOX therapy in the brain is due to the indirect action of the drug, because it is not able to cross the blood–brain barrier. DOX raises circulating levels of tumour necrosis factor (TNFα), which can cross this barrier, reaching and activating glial cells to initiate the local production of TNFα and raise its circulating

levels. This TNFα induces the local generation of reactive oxigen species (ROS) through nitric oxide synthase induction, and therefore intensifies the oxidative stress responsible for brain injury (16). Molecular mechanisms of DOX account for both its anti-cancer and its toxic effects (in the heart, brain, kidney, etc.). DOX acts at two fundamental levels: altering DNA and producing free radicals; in this respect, too, other mechanisms have been studied (5).

Generation of free radicals during metabolism of doxorubicin

The production of free radicals and oxidative stress is closely involved with DOX action, regarding both anti-tumour and toxic effects. There are four different modes of free radical production by DOX:

(**1**) Production of semiquinone: DOX is transformed into a semiquinone free radical through electron reduction by various NAD (P)H-dependent reductases in the complex I of the electron transport chain (cytochrome P-450 reductase). This semiquinone reacts with molecular oxygen to produce the superoxide radical ($O_2^{\cdot -}$) and it converts DOX into quinone. This quinone–semiquinone cycle generates large amounts of $O_2^{\cdot -}$, which subsequently give rise to ROS and RNS species such as hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$) or peroxynitrite ($ONOO^-$) (15, 16).

(**2**) Activation of NAD(P)H oxidases (NOXs): DOX activates NOXs, which give rise to free radicals that participate in activating the apoptotic pathway in cardiac cells (19). NOX activation may generate $ONOO^-$ through the mitochondrial production of ROS as $O_2^{\cdot -}$ and the reaction with nitric oxide (20). $ONOO^-$ also activates matrix metalloproteinases (MMPs); these proteases have been implicated as a major mechanism of the $ONOO^-$ dependent cardiotoxicity of DOX (21).

(**3**) DOX free radicals come from a non-enzymatic mechanism that involves reactions with iron. For example, Fe^{3+} reacts with DOX in a redox reaction after which the iron atom accepts an electron and a Fe^{2+}-DOX free radical complex is produced. This iron-adriamycin complex can reduces oxygen to H_2O_2 and other ROS (22, 23).

(**4**) Non-enzymatic mechanism: DOX interferes with non-enzymatic metabolic reactions in which iron is involved, and leads to ROS production. Thus, the DOX semiquinone, $O_2^{\cdot -}$, and H_2O_2, can promote the release of iron from ferritin and cytoplasmic aconitase, thus altering iron metabolism. Subsequently, iron can react with DOX and subsequently produce $HO^{\cdot}$ (14, 16).

(**5**) Products from the metabolism of DOX: this metabolism leads to ROS production. On the one hand, the side chain carbonyl group of the carbon 13 in DOX is converted into a hydroxyl group by aldoketo reductases, giving rise to a secondary alcohol (doxorubicinol), which can release iron from cytoplasmic aconitase, disturbing the iron metabolism and, therefore, causing oxidative stress. On the other hand, it can be metabolised into a lipophilic aglycone capable of dif diffusing through the mitochondrial membrane and accumulating within it. This aglycone is the starting point for several reactions that release electrons, producing ROS and disturbing the functional integrity of the respiratory chain (16). All the oxidative mechanisms described above are triggered by DOX to induce cancer cell death and toxic effects in cardiac myocytes. Moreover, it is important to take into account that the heart is very rich in mitochondria. DOX has the ability to modify the chemical composition, structure and function of biological membranes, mainly at the mitochondrial level, fundamentally due to the

peroxidation generated by DOX (24-26). It has been reported that phospholipid peroxidation induced by DOX can cause an exchange of mitochondrial and microsomal cholesterol with exogenous pools (24). The mitochondria contain a phospholipid, cardiolipin, in their inner membrane, and DOX has a high affinity for this cardiolipin, which results in the accumulation of DOX inside cardiac cells (15, 17). This effect may be enhanced by a highly unsaturated diet, producing a cardiolipin that is very rich in highly peroxidisable unsaturated fatty acids (26). In 2007, berthiaume and Wallace reported a gene expression profile in the heart of male rats treated with 6 weekly injections of 2 mg/kg DOX followed by a 5-week drug free period. Several pathways are closely related to mitochondria, including glycolysis and fatty acid metabolism, which supports the hypothesis that these organelles are key targets in DOX toxicity.

The same authors (27) also reviewed the importance of the mitochondrion in DOX cardiotoxicity, this being an important target of this chemotherapeutic drug, which induces ROS production due to the DOX redox cycling at complex I of the electron transport chain. This oxidative damage can impair both short and long term mitochondrial functioning, causing reduced energy production, oxidation of the mitochondrial DNA and loss of mitochondrial membrane potential by generating mitochondrial permeability transition (MPT) pores. It has been suggested that the adenine nucleotide translocator may be the principal component of those MPT pores, and the concentration of this protein has been shown to diminish after DOX administration, thus aggravating mitochondrial dysfunction (28). In addition to these events, a decrease in other mitochondrial components such as coenzyme Q (CoQ) has been reported, as a consequence of the oxidative stress associated with the administration of DOX (25).

Mechanisms related to alterations of DNA

Thus, it has been reported that DOX leads to direct oxidative injure to DNA (29, 30) and generates lipid peroxidation (25, 26, 31).

Topoisomerase II is likely to be one of the primary targets for the activity of anthracycline antibiotics (32). The induction by DOX of strand breaks in the DNA of L1210 leukemic cells was described more than 20 years ago (33). These strand breaks were protein-associated and slowly and incompletely repaired after removal of cells from the presence of the drug. Tewey et al. (1984) (34) have shown that topoisomerase II as the target enzyme for DOX and demonstrated that the subunits of the homodimeric enzyme remain locked onto the 5 end of the DNA molecule after completing the cleavage reaction. The capacity of DOX to inhibit DNA synthesis has been proposed as a mechanism of action of DOX (32). This mechanism may be related to DNA intercalation or inhibition of DNA polymerase activity (35). It is possible that this effect may be related to signalling events of growth arrest and p53 function (36). Another way of action of DOX through alterations in DNA is the induction of enzymatic or chemically activated DNA adducts (37), and DNA cross-linking (38).

Interference with DNA strand separation and helicase activity has also been postulated as mechanisms of action for DOX. It has been demonstrated that low concentrations of DOX interfere with DNA unwinding in MCF-7 breast tumour cells. This finding could potentially be related to the induction of DNA cross-links or to drug effects at the level of helicases (32).

Such genetic disturbances, together with the activation of tumour suppressor p53, and other additional mechanisms, are responsible for the apoptosis induced by DOX. Among those additional mechanisms, DOX can trigger apoptosis by producing ceramide (which prompts apoptosis by activating p53 or other downstream pathways such as JNK), the degradation of Akt by serine threonine proteases, the mitochondrial release of cytochrome c, increased FasL (death receptor Fas/CD95 ligand) mRNA production (14, 15, 17, 39), and a greater production of free radicals (16).

Tumor suppressor protein p53 occupies a pivotal position in maintaining genomic integrity (40). In response to celular stresses that lead to DNA damage, wild-type p53 orchestrates the transcription of numerous genes and directs cells to cell cycle arrest, senescence, or apoptosis via differential activation of target genes (41), preventing the propagation of damaged DNA (42). One of the most important questions in the study of p53 is how p53 determines a specific cellular outcome (e.g., selecting cell cycle arrest between senescence and apoptosis) via selectively regulating certain groups of target genes. Current knowledge shows that various effectors, including proteins and even noncoding RNAs (such as Myc (43), hCAS/CSE1L (44), Hzf (45), and miR-34 (46), can play a role in selective transactivation of p53 target genes that leads to different cellular outcomes.

Given that both ROS and p53 participate in multiple celular processes, there should be interactions between ROS and p53 and intersections between their signaling pathways. A microarray analysis of H_2O_2-treated human cells identified one-third of the 48 highly H_2O_2-reponsive genes as targets of p53 (47). Though it is generally recognized that oxidative stress is associated with p53-dependent cell cycle arrest, DNA repair, and apoptosis, a clear understanding of the mechanisms of the interactions between ROS and p53 is still elusive.

Metabolism of calcium and induced-DOX apoptosis

Many studies have reported alterations in calcium metabolism (mainly calcium overload) after DOX treatment (48). These alterations include increase in intracellular Ca^{2+}, accumulation of calcium in ventricular myocardium, and Ca^{2+} inclusions in mitochondria, Ca^{2+} transport abnormalities in cardiac tissue and alterations in the Ca^{2+} release function of the sarcoplasmic reticulum by effects on the Ca^{2+}-ATPase and the Ca^{2+} release channel.

Myocardial mitochondria also play an important role in the physiological regulation of the intracellular homeostasis of calcium ions by either sequestering or releasing Ca^{2+} (49). For this purpose, mitochondria and cardiomyocyte membranes are equipped with a complex array of Ca2+ transporters. Ca^{2+} is a key regulator of muscle contraction and of important mitochondrial enzymes such as pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and ATP synthase (50, 51). Doxorubicin interferes at various levels with the mitochondrial membrane and furthermore induces an influx of Ca^{2+} from the extracellular compartment into the cardiomyocytes (52). Alterations in mitochondrial Ca^{2+} transport in heart have been reported to range from inhibition of uptake by mitochondria upon exposure to doxorubicin in vitro to stimulation of uptake in rabbits after repeated DOX exposure . It is not known whether the alterations in myocellular Ca^{2+} homeostasis are primary or secondary to the respiratory chain dysfunction in chronic DOX cardiomyopathy. In vitro models suggest that the DOX-induced Ca^{2+} uptake by cardiomyocytes and their mitochondria inhibits

Complexes I and IV of the respiratory chain and enhances ROS generation, effects which may subsequently limit ATP production (53). These data are supported by the observation that decreased cellular ATP levels and thus mitochondrial dysfunctions were preceded by increases in mitochondrial Ca^{2+}. These studies, however, were carried out in vitro and therefore more closely reflect acute rather than chronic doxorubicin cardiotoxicity. We therefore aimed to investigate the effects of DOX on the mitochondrial respiratory chain and Ca^{2+} homeostasis in a model which closely mimics the chronic course of the patients (3). Heart failure may lead to the accumulation of calcium inside cardiac cells, making the described increase in Ca^{2+} levels a consequence of DOX action more than a cause. The fact that the multi-drug resistance pump frequently mediates resistance to the anthracyclines suggests the concept that DOX must enter the cell to express its toxicity. In fact, cell membrane and mitochondrial membrane have been related to DOX action (32).

There is extensive evidence that treatment with DOX induces apoptosis (38). However, apoptosis appears only at low concentrations of the drug, which supports the hypothesis that elevated concentrations of anthracyclines may kill the tumour cell through nonphysiological mechanisms (32). Another proposed mechanism is the formation of DOX metabolites.

Use of antioxidant in the prevention of DOX-induced cardiotoxicity

DOX increases cardiomyocyte susceptibility by reducing SOD activity and therefore reducing the ability of cardiac cells to inactivate ROS (54). Oxidative stress also has been shown to cause depolarization of the mitochondrial membrane, leading to programmed cell death (55).

Due to the great importance of DOX in chemotherapy for the treatment of many types of cancer, researchers have expended great efforts trying to prevent or attenuate the side effects of DOX administration. In this sense several strategies have been followed as dosage optimisation, synthesis and use of analogues or combined therapy. No promising results have been found with different routes and schedules of drug delivery (56, 57) and the use of several DOX analogues available clinically did not show stronger antitumor efficacy or lower toxic effects than DOX (58). In relation to combined therapy, although antitumor action of DOX may be mediated by a wide number of mechanisms, free radical production is among the main causes of cardiotoxicity mediated by this drug. This fact allows the assay of strategies trying to reduce the toxic effects of DOX without interference with its antitumor properties. The most immediate approach has been the combination of the drug delivery together with an antioxidant in order to reduce oxidative stress (59).

Vitamin E (α-tocopherol) has a high antioxidant capacity and plays a fundamental biologic role, especially in protecting cells and tissues from oxidative damage, and membrane lipid and lipoprotein peroxidation (15, 17). In general, preclinical studies in rodents have shown that oral vitamin E tends to increase antitumour actions and protects against the toxic effects of DOX (15); nevertheless, it has been reported that both vitamins E and C can increase the expression of P-glycoprotein and of hypoxia inducible factor-1alpha in Nox-1 overexpressing prostate tumour cells (60). Therefore, these vitamins might fortify resistance to chemotherapy, although this proposal needs to be more extensively studied. As a beneficial agent, vitamin E has been found to lengthen the life span of laboratory animals and to diminish the weight loss provoked by

chemotherapy. Moreover, this antioxidant can protect from both acute and chronic cardiotoxicity caused by DOX, and it increases antioxidant capacity in the heart (15).

Vitamin C (ascorbic acid) is an effective water soluble antioxidant against lipid peroxidation, scavenging ROS in the aqueous fraction before these molecules can give rise to lipid oxidation (61). An intervention review by van Dalen et al. (2008) showed that none of the individual studies carried out demonstrated a cardioprotective effect by vitamin C combined with vitamin E, mainly administered by oral supplementation (62).

Carotenoids such as β-carotene can reduce the lipid peroxidation associated with DOX and augment the anti-tumour effect of this drug (63). Oral supplementation with lycopene (5 mg/kg/day for 7 weeks), the carotenoid presenting the most powerful antioxidant activity, has demonstrated a cardioprotective effect at the myocyte level in rats treated with DOX (4 mg/kg) intraperitoneally by weeks 3, 4, 5 and 6, but it fails to prevent DOX- induced cardiac dysfunction (1). Moreover, it has been reported that a tomato oleoresin supplement containing lycopene (95%), all-trans-β-carotene (5%), and 13-cis-β-carotene (1%), reduces cardiomyocyte oxidative DNA damage caused by DOX in rats (64). The same authors reported that DOX maintains levels of lycopene in the myocardial tissue of rats, and at the same time it raises the antioxidant capacity of this tissue. This suggests DOX has an antioxidant more than a pro-oxidant effect (65). Vitamin A also has a protective dose-dependent effect against the chromosomal aberrations induced by DOX in rat bone marrow cells, 15 μg/kg being the most effective dose, whereas 30 μg/kg was found to be clastogenic (66).

Coenzyme Q (CoQ), or ubiquinone, plays a critical role in the mitochondrial respiratory chain, acting as a redox link between flavoproteins and cytochromes, being an essential component in extramitochondrial redox chains. Its concentration in blood and tissues depends on biologic requirements, endogenous biosynthesis, and of course the dietary intake (17). Due to the high presence of CoQ in the mitochondria, its concentration reflects the cellular content of mitochondria among different tissues, being greater in the heart than in the liver (fivefold more), kidney, pancreas, spleen (10-fold more), and skeletal muscle (5% more) (67). Adriamycin induces cardiotoxicity by lipid peroxidation in cardiac myocytes, reduces the content of CoQ10 in mitochondrial membranes, and inhibits the mitochondrial biosynthesis of CoQ10 as well as respiratory chain CoQ10-dependent enzymes (62). Similar effects have been found in rats where the plasma and mitochondria levels of CoQ10 and CoQ9, respectively, were sharply decreased by the oxidative stress generated by DOX (25).

Flavonoids are characterized by high antioxidant power, and have been considered potential protectors against the chronic cardiotoxicity associated with DOX (17). This protective effect of flavonoids is closely related to their antioxidant, iron chelating (68) and carbonyl reductase 1 (CBR1)-inhibitory properties (69). The proposed mechanism involving iron chelating and antioxidant activities involves two steps: (1) iron is chelated by the flavonoid; (2) ROS production is quickly scavenged by flavonoids at the place of generation; such a concept has been termed site-specific scavenging (68). The semisynthetic flavonoid 7-monohydroxyethylrutoside has been extensively studied as a good cardioprotective compound, both in preclinical (intraperitoneally administered in mice) (70) and in clinical trials after intravenous injection. This flavonoid inhibits negative cardiac effects in a dose-dependent manner, in accordance with the essential properties of all flavonoids, i.e. their iron chelating and antioxidant characteristics. Other flavonoids, such as catechins, have cardioprotective properties at low doses,

exhibiting an iron chelating activity. These catechins also have beneficial properties for the liver, and Kalender et al. (2005) reported that catechin (200 mg/kg/week for 6 weeks by intraperitoneal injection) depressed malondialdehyde, glutathione peroxidase and catalase activities in rats against DOX- induced hepatotoxicity (71). Two fundamental biochemical mechanisms of flavonoids can increase the anti-tumour capacity of DOX when they are administered jointly: (1) the inhibition of the intracellular metabolism of the drug; (2) the blocking of intracellular drug eliminating mechanisms. In previous in vitro studies, it has been shown that green tea polyphenols such as caffeine and catechins such as epigallocatechin gallate or epigallocatechin can enhance DOX-induced anti-tumour activity and increase DOX concentration in tumours by inhibiting its efflux (17).

Conclusions

Cellular damage induced by DOX is mediated by the formation of an iron-DOX complex that generates free radical, wich, in turn, causes severe damage to the plasma membrane and interferences with the cytoskeltal structure. Due to the low levels of antioxidant defenses, hert is particularly vulnerable to injury by DOX-induced ROS. DOX can induce structural changes and death in the myocardium of treated patients, and the extent of structural damage has been correlated with early cardiac dysfunction.

Therapeutic strategies, designed to augment cellular endogenous antixoidant systems has been identified as a promising approach to combat oxidative stress-associated disease condition.

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