Universiteit Leiden
The Netherlands

Anthracycline-induced cardiotoxicity, a pathophysiology based approach for early detection and protective strategies

Broeyer, F.J.F.

Citation
Broeyer, F. J. F. (2012, January 17). *Anthracycline-induced cardiotoxicity, a pathophysiology based approach for early detection and protective strategies*. Retrieved from https://hdl.handle.net/1887/18360

Version:	Corrected Publisher's Version
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Note: To cite this publication please use the final published version (if applicable).

CHAPTER 1

Introduction and outline of the thesis

Cardiac effects of anthracyclines

The first anthracycline, daunorubicin, was originally isolated from the *S. peucetius* in 1957.(1) Since then, numerous analogues have been developed, of which doxorubicin (Adriamycin®) is still the most commonly used (figure 1).(2) Doxorubicin is used in the treatment of a wide range of malignancies, including breast cancer, ovarian cancer, leukemia and sarcomas.(3)

Soon after the introduction of doxorubicin, cardiotoxic side-effects were noted.(1;4) These effects are commonly divided into acute, sub-acute and chronic effects.(5) The acute effects consist of rhythm disturbances and myocarditis and occur almost instantaneously after doxorubicin administration. The sub-acute and chronic effects develop after at least 3 months, but can also become apparent years later and may lead to congestive heart failure (CHF). The latter types of toxicity are mainly dose-dependent and vary between < 1% for doses up to 450 mg/m^2 and 47% for doses over 700 mg/m^2.(6) Other risk-factors include age and gender.(4) Commonly used concomitant therapies in the treatment of cancers, like radiation and Herceptin, are also known to increase the occurrence of doxorubicin induced cardiotoxicity.(7)

Molecular mechanisms of cardiac effects of anthracyclines

The cardiotoxic effects of doxorubicin are probably mediated by the induction of apoptosis via free radical mediated mechanisms, but deregulation of intracellular Ca-homeostasis seems to play a role as well.(8) This is different from the anti-tumor effect, which is mainly regulated via interference with DNA replication, alkylation and cross-linking, RNA transcription and inhibition of topoisomerase II.(3)

Oxidative stress and apoptosis

Oxidative stress is defined as 'a disturbance in the prooxidant-antioxidant balance in favor of the former, leading to potential damage'.(9) This imbalance can either be caused by an excess of reactive oxygen species (ROS) or a diminished amount of antioxidants such as superoxide dismutase (SOD), catalase, vitamins. Oxidative stress is involved in the pathophysiology of a wide range of diseases and an excess of ROS also plays an important role in anthracycline-induced cardiotoxicity. After administration of doxorubicin, free radicals are formed by one-electron addition to its quinone moiety, which quickly regenerates to its original structure by reducing oxygen to the superoxide anion ($O_2^{\bullet -}$) (figure 2).These reactive oxygen species induce apoptosis, both via the extrinsic (Fas-mediated) and the intrinsic (mitochondrial) pathway.(10-13) Cardiomyocytes are especially vulnerable to doxorubicin-induced apoptosis, as they contain low levels of ROS scavenging enzymes.(14) Although the role of apoptosis in doxorubicin-induced cardiotoxicity is well-established *in vitro*, it remains uncertain whether this mechanism is responsible for the chronic cardiac toxicity.(8)

Secondary alcohol metabolites

Doxorubicin may also cause cardiac myopathy by the formation of its secondary alcohol metabolites such as doxorubicinol (DOXOL). Two mechanisms for its cardiotoxicity have been postulated: interference with intracellular Ca handling, and indirectly by disrupting iron metabolism, mainly by switching off the Iron Regulatory Protein 1 (IRP-1).(15) Direct evidence that the alcohol metabolites of doxorubicin play a relevant role in cardiotoxicity is based upon the observation that animals lacking or overexpressing the gene coding for the enzyme that catalyses the conversion of doxorubicin to DOXOL show decreased and increased cardiotoxicity respectively. The involvement of secondary alcohol metabolites is further supported by the observation that taxanes that stimulate

the formation of doxorubicinol aggravate the cardiotoxicity of doxorubicin.(16;17) In keeping with these findings is the relationship between the extent of formation of secondary alcohol metabolites of anthracycline analogue *in vitro* and the clinically observed cardiotoxicity.(8;18)

Iron-mediated toxicity

Iron can intensify the damage induced by ROS and induce the formation of hydroxyl (.OH) radicals via the Haber-Weiss reaction ($O_2^{\bullet -} + H_2O_2 \rightarrow OH^{\bullet} + OH^- + O_2$). This reaction can only occur when an intracellular pool of free iron is present.(19) To date, it has not been completely elucidated how such a pool of free iron is formed within the cardiomyocyte. Most of the iron is stored in the iron-storage protein ferritin. Conflicting evidence exists with regard to iron release from ferritin in the presence of doxorubicin. Earlier studies showed that the presence of $O_2^{\bullet -}$ and the semiquinone of doxorubicin enabled Fe^{2+} release from ferritin.(20-22) However, subsequent studies paradoxically demonstrated that doxorubicin favors accumulation of iron in ferritin by causing post-transcriptional changes to ferritin resulting in a decreased ability to release Fe^{2+}.(23;24) It is hypothesized that these mechanisms can be both protective and unfavorable, as iron within ferritin is not available for free radical reactions. However, free iron deficiency hampers several intracellular processes, such as DNA synthesis.(19)

Doxorubicin and its metabolite doxorubicinol influence iron metabolism in a different way as well, as they interfere with the regulation by Iron Regulatory Proteins (IRPs).(15) Cellular iron regulation is partly dependent on regulation by IRPs which can bind to the iron-responsive regions (IRES) present on 5'- or 3' untranslated regions mRNA of, among others, ferritin and the transferrin receptor (TfR). Binding IRP to IRE results in increased intracellular iron uptake and decreased iron storage of iron in ferritin. Two related IRPs have been identified in humans, namely IRP1 and IRP2. IRP1 switches from its active apo form, which is capable to bind IRES, to its inactive holo form in the presence of abundant intracellular iron. This switch is controlled via a 4Fe-S cluster present within IRP1 (figure 3).(25;26)

IRP2 shares extensive sequence homology with IRP1, but lacks the 4Fe-S cluster and its activity is regulated by protosomal degradation in the presence of intracellular iron.(25;26) Doxorubicin and doxorubicinol interfere with this mechanism via several distinct mechanisms. It has been described that doxorubicin (and doxorubicinol) irreversibly inactivated IRP1 (formation of a null protein), resulting in decreased IRP1-RNE binding.(27) Further investigations revealed that low (sub-clinical) concentrations of doxorubicin actually increased IRP1-RNE interaction, while higher concentrations of doxorubicin indeed led to the formation of a null protein.(28;29) In contrast with the "null protein"-theory, a subsequent study showed that complexes of doxorubicin Fe and Cu reversibly decreased IRP1-RNE binding by formation of disulfide complexes.(13) The influence of doxorubicin on IRP2-RNE binding is less extensively investigated. However, doxorubicin also appears to decrease IRP2-RNE binding.(29;30) It thus seems that doxorubicin favors iron sequestration over iron uptake by diminished IRP1- and IRP2-RNE binding.

In summary, the pathophysiology of doxorubicin is an accumulation of several processes, in which the formation of free radicals and the disturbance of iron metabolism are key features.

Endogenous defense mechanisms against cardiac effects of anthracyclines

At present, several mechanisms to protect against free radical induced cardiotoxicity have been identified. These include prooxidant-reducing proteins such as transferrin and haptoglobin, heat-shock proteins and antioxidants, such as vitamin C and E, catalase and superoxide dismutase (SOD). SOD catalyzes ($O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2$) the reaction in which $O_2^{\bullet -}$ is converted to hydrogen peroxide, which is further degraded by catalase to water. ($2H_2O_2 \rightarrow 2\ H_2O + O_2$). Three isoforms of SOD have been identified: cytosolic SOD (SOD1), mitochondrial SOD (SOD2) and extracellular SOD (SOD3). The intracellular forms are more abundantly present.

Detection methods for cardiac side effects of anthracyclines

Detection of anthracycline-induced cardiotoxicity is difficult, as a clinically relevant decline in left ventricular function often appears late after the administration of anthracyclines. Because of the impact of the impaired cardiac function, several detection methods have been investigated and evaluated to detect anthracycline-induced cardiotoxicity as early as possible.

ENDOMYOCARDIAL BIOPSY Endomyocardial biopsy was often used until the 1980s for the detection of anthracycline-induced cardiotoxicity.(31-33) After exposure to anthracyclines, highly specific histopathological changes occur in the myocardium, including extensive depletion of myofibrillar bundles, myofibrillar lysis, distortion and disruption of z-lines, mitochondrial swelling and swelling and disruption of the sarcoplasmatic reticulum, leading to intramyocyte vacuolization.(34-36) These changes are dose-dependent and occur scattered throughout the myocardium. (36) Endomyocardial biopsy is the most sensitive and specific method to detect anthracycline-induced cardiotoxicity. However, it is largely abandoned now, because of the lack of experience in obtaining and assessing the biopsies and the fact that it is a highly invasive procedure.(31-33)

MEASUREMENT OF LEFT VENTRICULAR FUNCTION Another commonly used method to assess cardiac function after treatment with anthracyclines is by determination of left ventricular systolic and diastolic function by either multigated radionuclide angiography (MUGA) or cardiac echography. With a MUGA scan, gamma radiation produced by 99Technetium-labeled erythrocytes is measured and used to calculate several cardiac indices for systolic and diastolic function, such as left ventricular ejection fraction (LVEF).(37) Radionuclide assessment of LVEF is widely used to determine left ventricular function in cardiac disease. As several studies have shown that a decline in nuclear LVEF is indeed predictive (sensitivity varies between 55% and 100%) for future congestive heart failure in patients using anthracyclines, it is currently regarded as the gold standard.(38-44)

Also echographic assessment of LVEF has been used to assess anthracycline-induced cardiotoxicity. Literature shows that, when appropriate techniques are used, assessment of LVEF with this method is comparable to LVEF assessment with radionuclides. (45-48)

A disadvantage of both nuclear and echographic determination of the LVEF is that it is unclear if it is feasible for the early detection of cardiotoxicity, as the decline in LVEF commonly occurs late in the pathophysiological process and is often insidious. (49-51) However, other reports show that even small early (a change of 4% in ejection fraction) changes in LVEF measured with echocardiography, as well as with radionuclide methods may be predictive for the occurrence of anthracycline-induced cardiotoxicity.(52-54)

In the past few decades, heart failure with preserved ejection fraction has become increasingly important and has also been associated with significant morbidity and mortality.(55;56) Administration of anthracyclines also impairs diastolic function, even in the absence of a declined left ventricular ejection fraction. (57-63) It has therefore been suggested that diastolic dysfunction could precede an ensuing decline in LVEF and may be useful as a marker for anthracycline-induced cardiotoxicity.

Biochemical markers - cardiac troponins and natriuretic peptides

In cardiovascular disease, including anthracycline-induced heart failure, an extensive amount of biomarkers has been studied for the detection of myocardial injury, including creatine kinases, cardiac troponins and natriuretic peptides.

Troponins are thin-filament associated complexes that are involved in the regulation of the actin-myosin cross-bridges of striated muscles and consist of three subunits: troponin T, C and I.(64) Cardiac troponin T and I are both highly sensitive and specific markers for myocardial injury.(65) Both markers are established as diagnostic and prognostic tools in acute coronary syndromes. (66) Cardiac troponins have also been suggested as early markers for anthracycline-induced cardiotoxicity, albeit with ambivalent

results. (For an excellent review, see Germanakis et al.(67)) These contrasting findings can be related to many factors, including heterogenic study populations, variable cumulative anthracycline doses, and different study protocols with regard to type of assay and sampling time. Nevertheless, most reports show that at least some patients have detectable troponin, suggesting that it might be a prognostic marker in anthracycline-induced cardiotoxicity.

The family of natriuretic peptides consists of 3 distinct types: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). All natriuretic peptides share vasodilative properties and are involved in sodium and water homeostasis.(68) ANP is stored in granules inside cardiomyocytes and released in response to cardiac wall stress. The formation of BNP in response to cardiac wall stress is more complex; pre-proBNP is synthesized in the ventricular wall and subsequently cleaved via proBNP into its active form BNP and the inactive amino-terminal fragment NT-proBNP. As BNP is less sensitive for transient changes in hemodynamics, such as the administration of infusion fluids, it is a better marker for cardiovascular disease, such as heart failure, than ANP.(68) Indeed, both BNP and NT-proBNP are known to be increased in heart failure and both markers are widely used as independent risk factors for cardiovascular events. Most studies have shown that elevated BNP and NT-proBNP levels correlate well with echocardiographic and/or radionuclide parameters of myocardial dysfunction.(69) CNP is mainly produced by the endothelium and its role in the pathophysiology of heart disease is yet to be established.(70) It has been reported that after administration of anthracyclines, concentrations of circulating natriuretic peptides increase. Especially persistently elevated (NT-pro)BNP levels have prognostic value.(71) However, NT-proBNP has also been suggested as possible early marker for the evaluation of anthracycline-induced cardiotoxicity in children,(71) and adults.(this thesis) A disadvantage however is that BNP (and to a lesser extent also NT-proBNP) levels are subject to biological (day-to-day) variation which make them less suitable for monitoring disease progression unless strict protocols are followed.(72)

ELECTROCARDIOGRAPHY Electrocardiography is widely used for the evaluation of cardiac function, and is useful for the diagnosis of cardiac ischemic diseases, congestive heart failure and arrhythmias. Several clinical studies have related prolongation of the QT-interval to anthracycline administration.(73-76) As anthracyclines are not known to influence cardiac ion channels, these prolonged QT-intervals may be related to a disturbed repolarization due to myocardial injury. Although no relation between the degree of QT-prolongation and cardiac disease has been shown in patients treated with anthracyclines, it is known that prolonged QT-intervals are associated with increased mortality in patients with heart failure.(77) Other evidence that QT-prolongation might be an appropriate marker for cardiac injury is suggested by the commonly used preclinical model for anthracycline-induced cardiotoxicity. In mice, adriamycin induces ST-prolongations that can be abolished by concomitant administration of the clinically effective protective compound dexrazoxane.(78) Some investigators also related an increased QT-dispersion, which reflects the regional differences in repolarization, to exposure to anthracyclines.(79-81) Increased QT-dispersion has been related to increased cardiac mortality in various clinical conditions.(82) However, more recent studies indicate that QT-dispersion is an unreliable predictor of cardiac events in general(83;84), making it unlikely that QT-dispersion will prove to be a suitable marker for anthracycline-induced cardiac injury. Finally, changes in heart rate variability (HRV), which reflects changes in autonomic regulation of circulatory function, have also been described after anthracycline administration(85;86), but a subsequent report failed to confirm these results.(49) rendering the value of this measure questionable.

Protective strategies

Several protective strategies have been suggested in order to diminish the cardiotoxicity by anthracyclines, including less toxic compounds, improved dosage schedules and the concomitant administration of protective compounds.

LESS TOXIC ANTHRACYCLINES Numerous presumed less toxic analogues have been developed, of which only epirubicin

and idarubicin are used in clinical practice. Although epirubicin is less cardiotoxic than doxorubicin, this advantage is clinically less significant as higher doses are needed to achieve similar anti-tumor efficacy compared to doxorubicin, thereby offsetting the favorable cardiotoxic profile. The data for idarubicin are contradictory and larger trials are needed to assess if this analogue indeed has a lower incidence of cardiotoxicity.(8) Some advances have been made with the development of liposome-encapsulated doxorubicin, these suggest a favorable cardiotoxic profile. However, only limited efficacy data are available and treatment costs are relatively high. The value of this compound thus remains to be established.(2)

DIFFERENT DOSING STRATEGIES Traditionally, anthracyclines are administered as bolus infusion over a maximum of approximately 60 minutes. Soon after their introduction it was suggested that a prolonged infusion period (up to 96 hours) could reduce cardiotoxicity.(87) Since then, over 30 trials have compared the occurrence of (sub-)clinical cardiotoxicity after bolus injection with prolonged infusions. (For a review, see (88) According to a meta-analysis, the occurrence of clinical heart failure is significantly lower in patients receiving anthracyclines with an infusion duration of six hours or longer as compared to bolus infusion (RR = 0.27; 95%CI 0.09 to 0.81, P = 0.02).(88) Although it seems that the anti-tumor efficacy is not hampered by slow infusion, these trials were mainly performed in patients with metastasized disease and the follow up period was not clearly specified. Should it be proven, however, that modification of dosing schedules does not to impair the intended anti-tumor effects of anthracyclines while avoiding the untoward cardiac effects, this might prove a feasible future strategy.

PROTECTIVE AGENTS Based on the (presumed) pathophysiological mechanism, protective compounds were developed that should be administered concomitantly with the anthracycline-containing chemotherapy. As ROS-overload is a major pathophysiological mechanism it is logical that free radical scavengers like N-acetylcysteine, coenzyme Q10, vitamin E and C were evaluated as protective agents.(89-92) Indeed, animal studies with these compounds showed promising results, but evidence of protective effects in humans could not be not demonstrated.

Blocking the renin-angiotensin system (RAS), either by ACE inhibitors or ATII receptor blockers, improves the outcome of patients with systolic heart failure.(93) In the treatment of anthracycline-induced cardiac failure, treatment with ACE-inhibitors was efficacious, too.(94-97) This suggests that concomitant administration of RAS-inhibiting agents may be beneficial. This hypothesis was supported by animal studies showing that modulation of the RAS could prevent against anthracycline-induced cardiotoxicity.(89;98-103) It appears that in clinical practice, too, ACE inhibitors and ATII-antagonists are beneficial in patients treated with anthracyclines.(104;105) However, these trials were performed in small patient populations and had several methodological shortcomings, so additional research is needed to confirm whether or not ACE-inhibitors/ATII antagonists can be considered as protective.

The only compound that has a proven efficacy against anthracycline-induced cardiac failure is the iron chelator dexrazoxane.(106) This compound is capable to chelate intracellular iron (complexes), thereby preventing the formation of free radicals.(107) However, the possible association of dexrazoxane with a higher risk for secondary malignancies and an increased occurrence of (serious) adverse effects limits its clinical use to patients with advanced (metastasized) tumors.(106)

Aims of the thesis

It is clear that there is a special need for markers that can be used to detect anthracycline-induced cardiotoxicity in an early stage and identify those patients at risk for the development of CHF.

The studies described in this thesis aim to, firstly, identify possible biomarkers and detection methods to identify anthracycline-induced cardiotoxicity early and secondly, identify new possible strategies to prevent anthracycline-induced cardiotoxicity. Chapter 2 comprises a pilot-study to identify biomarkers for course-to-course evaluation of

anthracycline-induced cardiotoxicity. In chapter 3, the effects of doxorubicin on the iron metabolism is discussed. A novel method to assess repolarization disturbances after anthracycline therapy is described in chapter 4. In chapter 5 and 6, the pharmacokinetics of a potentially novel protective compound lecithinized superoxide dismutase (PC-SOD) in healthy subjects are described. These data were used to design the study described in chapter 7. This study investigated the clinical efficacy of PC-SOD against anthracycline-induced cardiotoxicity in female breast cancer patients. It concludes with an overall discussion, conclusions and suggestions for further research (chapter 8).

Figure 1 Chemical structure of Doxorubicin

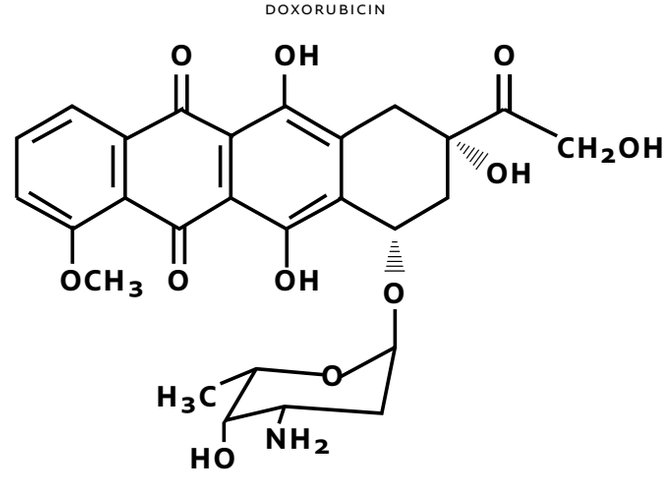


Figure 2 ROS generation after anthracycline administration

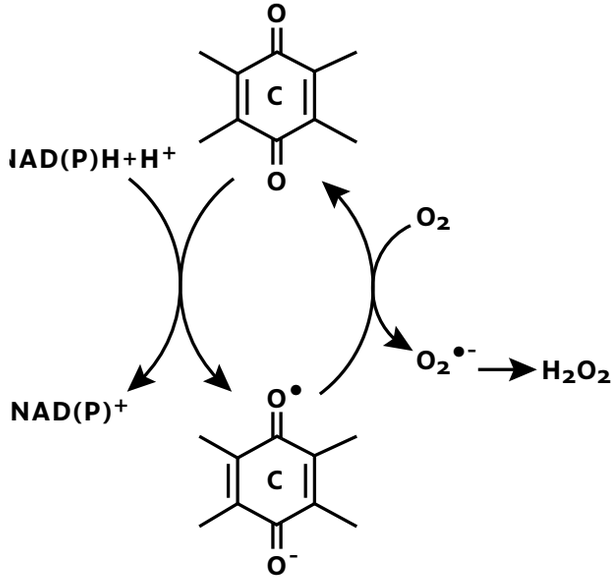
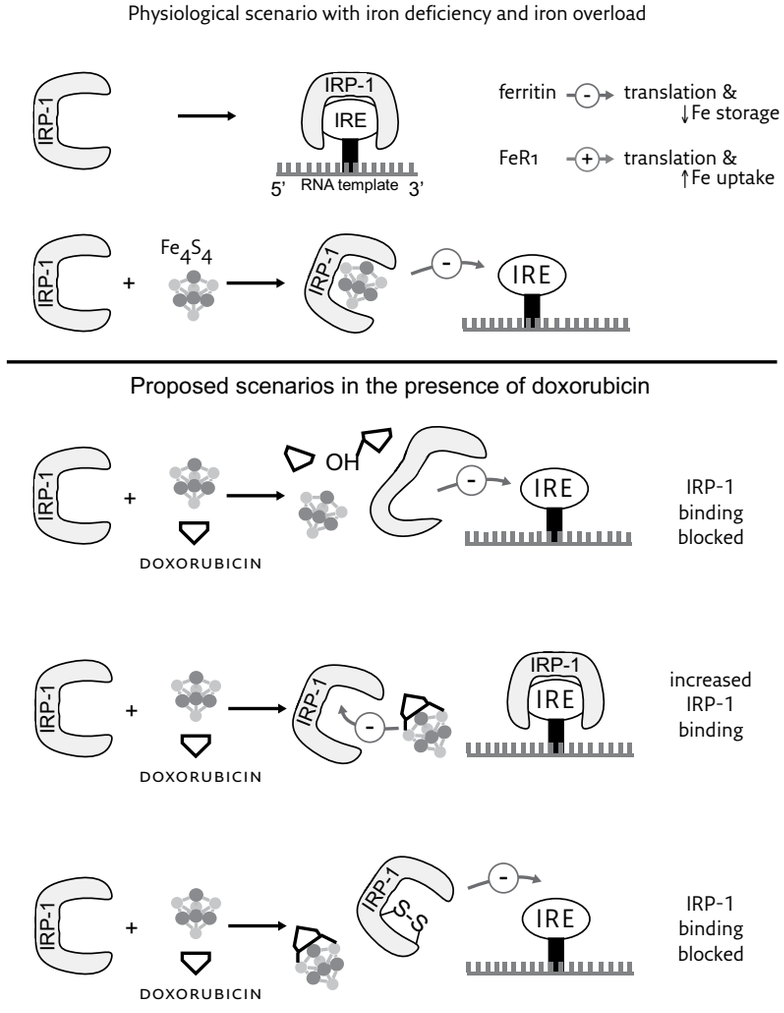


Figure 3 Proposed mechanisms of iron-mediated cardiotoxicity



1 Di Marco A, Gaetani M, Scarpinato B. Adriamycin (NSC-123,127): a new antibiotic with antitumor activity. Cancer Chemother Rep 1969 Feb;53(1):33-7.

2 van Dalen EC, Michiels EM, Caron HN, Kremer LC. Different anthracycline derivates for reducing cardiotoxicity in cancer patients. Cochrane Database Syst Rev 2010;(5):CD005006.

3 Gewirtz DA. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochemical Pharmacology 1999 Apr 1;57(7):727-41.

4 Von Hoff DD, Layard MW, Basa P, Davis HL, Jr., Von Hoff AL, Rozencweig M, et al. Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 1979 Nov;91(5):710-7.

5 Shan K, Lincoff AM, Young JB. Anthracycline-Induced Cardiotoxicity. Annals of Internal Medicine 1996 Jul 1;125(1):47-58.

6 Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. Cancer 2003 Jun 1;97(11):2869-79.

7 Singal PK, Iliskovic N. Doxorubicin-Induced Cardiomyopathy. The New England Journal of Medicine 1998 Sep 24;339(13):900-5.

8 Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev 2004 Jun;56(2):185-229.

9 Sies H. II. Oxidants and Antioxidants. Oxidative stress.London: Academic Press; 1991.

10 Kalivendi SV, Konorev EA, Cunningham S, Vanamala SK, Kaji EH, Joseph J, et al. Doxorubicin activates nuclear factor of activated T-lymphocytes and Fas ligand transcription: role of mitochondrial reactive oxygen species and calcium. Biochem J 2005 Jul 15;389(Pt 2):527-39.

11 Kotamraju S, Konorev EA, Joseph J, Kalyanaraman B. Doxorubicin-induced apoptosis in endothelial cells and cardiomyocytes is ameliorated by nitrone spin traps and ebselen. Role of reactive oxygen and nitrogen species. J Biol Chem 2000 Oct 27;275(43):33585-92.

12 Nakamura T, Ueda Y, Juan Y, Katsuda S, Takahashi H, Koh E. Fas-mediated apoptosis in adriamycin-induced cardiomyopathy in rats: In vivo study. Circulation 2000 Aug 1;102(5):572-8.

13 Yamaoka M, Yamaguchi S, Suzuki T, Okuyama M, Nitobe J, Nakamura N, et al. Apoptosis in rat cardiac myocytes induced by Fas ligand: priming for Fas-mediated apoptosis with doxorubicin. J Mol Cell Cardiol 2000 Jun;32(6):881-9.

14 Doroshow JH, Locker GY, Myers CE. Enzymatic defenses of the mouse heart against reactive oxygen metabolites: alterations produced by doxorubicin. J Clin Invest 1980 Jan;65(1):128-35.

15 Xu X, Persson HL, Richardson DR. Molecular pharmacology of the interaction of anthracyclines with iron. Mol Pharmacol 2005 Aug;68(2):261-71.

16 Salvatorelli E, Menna P, Cascegna S, Liberi G, Calafiore AM, Gianni L, et al. Paclitaxel and docetaxel stimulation of doxorubicinol formation in the human heart: implications for cardiotoxicity of doxorubicin-taxane chemotherapies. J Pharmacol Exp Ther 2006 Jul;318(1):424-33.

17 Perotti A, Cresta S, Grasselli G, Capri G, Minotti G, Gianni L. Cardiotoxic effects of anthracycline-taxane combinations. Expert Opin Drug Saf 2003 Jan;2(1):59-71.

18 Sawyer DB, Peng X, Chen B, Pentassuglia L, Lim CC. Mechanisms of anthracycline cardiac injury: can we identify strategies for cardioprotection? Prog Cardiovasc Dis 2010 Sep;53(2):105-13.

19 MacKenzie EL, Iwasaki K, Tsuji Y. Intracellular iron transport and storage: from molecular mechanisms to health implications. Antioxid Redox Signal 2008 Jun;10(6):997-1030.

20 Minotti G. Sources and role of iron in lipid peroxidation. Chem Res Toxicol 1993 Mar;6(2):134-46.

21 Monteiro HP, Vile GF, Winterbourn CC. Release of iron from ferritin by semiquinone, anthracycline, bipyridyl, and nitroaromatic radicals. Free Radic Biol Med 1989;6(6):587-91.

22 Thomas CE, Aust SD. Release of iron from ferritin by cardiotoxic anthracycline antibiotics. Arch Biochem Biophys 1986 Aug 1;248(2):684-9.

23 Corna G, Santambrogio P, Minotti G, Cairo G. Doxorubicin Paradoxically Protects Cardiomyocytes against Iron-mediated Toxicity: ROLE OF REACTIVE OXYGEN SPECIES AND FERRITIN. J Biol Chem 2004 Apr 2;279(14):13738-45.

24 Kwok JC, Richardson DR. Anthracyclines induce accumulation of iron in ferritin in myocardial and neoplastic cells: inhibition of the ferritin iron mobilization pathway. Mol Pharmacol 2003 Apr;63(4):849-61.

25 Andrews NC, Schmidt PJ. Iron homeostasis. Annu Rev Physiol 2007;69:69-85.

26 Hentze MW, Kuhn LC. Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide, and oxidative stress. Proc Natl Acad Sci U S A 1996 Aug 6;93(16):8175-82.

27 Minotti G, Recalcati S, Mordente A, Liberi G, Calafiore AM, Mancuso C, et al. The secondary alcohol metabolite of doxorubicin irreversibly inactivates aconitase/iron regulatory protein-1 in cytosolic fractions from human myocardium. FASEB J 1998 May;12(7):541-52.

28 Kotamraju S, Chitambar CR, Kalivendi SV, Joseph J, Kalyanaraman B. Transferrin receptor-dependent iron uptake is responsible for doxorubicin-mediated apoptosis in endothelial cells: role of oxidant-induced iron signaling in apoptosis. J Biol Chem 2002 May 10;277(19):17179-87.

29 Minotti G, Ronchi R, Salvatorelli E, Menna P, Cairo G. Doxorubicin irreversibly inactivates iron regulatory proteins 1 and 2 in cardiomyocytes: evidence for distinct metabolic pathways and implications for iron-mediated cardiotoxicity of antitumor therapy. Cancer Res 2001 Dec 1;61(23):8422-8.

30 Kwok JC, Richardson DR. Unexpected anthracycline-mediated alterations in iron-regulatory protein-RNA-binding activity: the iron and copper complexes of anthracyclines decrease RNA-binding activity. Mol Pharmacol 2002 Oct;62(4):888-900.

31 Mason JW, Bristow MR, Billingham ME, Daniels JR. Invasive and noninvasive methods of assessing adriamycin cardiotoxic effects in man: superiority of histopathologic assessment using endomyocardial biopsy. Cancer Treat Rep 1978 Jun;62(6):857-64.

32 Benjamin RS, Mason JW, Billingham ME. Cardiac toxicity of adriamycin-DNA complex and rubidazone: evaluation by electrocardiogram and endomyocardial biopsy. Cancer Treat Rep 1978 Jun;62(6):935-9.

33 Bristow MR, Mason JW, Billingham ME, Daniels JR. Doxorubicin cardiomyopathy: evaluation by phonocardiography, endomyocardial biopsy, and cardiac catheterization. Ann Intern Med 1978 Feb;88(2):168-75.

34 Torti FM, Bristow MM, Lum BL, Carter SK, Howes AE, Aston DA, et al. Cardiotoxicity of epirubicin and doxorubicin: assessment by endomyocardial biopsy. Cancer Res 1986 Jul;46(7):3722-7.

35 Billingham ME, Mason JW, Bristow MR, Daniels JR. Anthracycline cardiomyopathy monitored by morphologic changes. Cancer Treat Rep 1978 Jun;62(6):865-72.

36 Bristow MR, Mason JW, Billingham ME, Daniels JR. Dose-effect and structure-function relationships in doxorubicin cardiomyopathy. Am Heart J 1981 Oct;102(4):709-18.

37 Strauss HW, Zaret BL, Hurley PJ, Natarajan TK, Pitt B. A scintiphotographic method for measuring left ventricular ejection fraction in man without cardiac catheterization. The American Journal of Cardiology 1971 Nov;28(5):575-80.

38 Druck MN, Gulenchyn KY, Evans WK, Gotlieb A, Srigley JR, Bar-Shlomo BZ, et al. Radionuclide angiography and endomyocardial biopsy in the assessment of doxorubicin cardiotoxicity. Cancer 1984 Apr 15;53(8):1667-74.

39 Schwartz RG, McKenzie WB, Alexander J, Sager P, D'Souza A, Manatunga A, et al. Congestive heart failure and left ventricular dysfunction complicating doxorubicin therapy. Seven-year experience using serial radionuclide angiocardiography. Am J Med 1987 Jun;82(6):1109-18.

40 Alexander J, Dainiak N, Berger HJ, Goldman L, Johnstone D, Reduto L, et al. Serial assessment of doxorubicin cardiotoxicity with quantitative radionuclide angiocardiography. N Engl J Med 1979 Feb 8;300(6):278-83.

41 Gottdiener JS, Mathisen DJ, Borer JS, Bonow RO, Myers CE, Barr LH, et al. Doxorubicin cardiotoxicity: assessment of late left ventricular dysfunction by radionuclide cineangiography. Ann Intern Med 1981 Apr;94(4 pt 1):430-5.

42 McKillop JH, Bristow MR, Goris ML, Billingham ME, Bockemuehl K. Sensitivity and specificity of radionuclide ejection fractions in doxorubicin cardiotoxicity. Am Heart J 1983 Nov;106(5 Pt 1) :1048-56.

43 Ritchie JL, Singer JW, Thorning D, Sorensen SG, Hamilton GW. Anthracycline cardiotoxicity: clinical and pathologic outcomes assessed by radionuclide ejection fraction. Cancer 1980 Sep 1;46(5):1109-16.

44 Corapcioglu F, Sarper N, Berk F, Sahin T, Zengin E, Demir H. Evaluation of anthracycline-induced early left ventricular dysfunction in

children with cancer: a comparative study with echocardiography and multigated radionuclide angiography. Pediatr Hematol Oncol 2006 Jan;23(1):71-80.
45 Vourvouri EC, Poldermans D, Bax JJ, Sianos G, Sozzi FB, Schinkel AF, et al. Evaluation of left ventricular function and volumes in patients with ischaemic cardiomyopathy: gated single-photon emission computed tomography versus two-dimensional echocardiography. Eur J Nucl Med 2001 Nov;28(11):1610-5.
46 Folland ED, Parisi AF, Moynihan PF, Jones DR, Feldman CL, Tow DE. Assessment of left ventricular ejection fraction and volumes by real-time, two-dimensional echocardiography. A comparison of cineangiographic and radionuclide techniques. Circulation 1979 Oct;60(4):760-6.
47 Choragudi NL, Prakash AM, Sun Y, Prasad P, Chiaramida SA, Lucariello RJ. Comparison of echocardiography with technetium 99m-gated single photon emission computed tomography as diagnostic tools for left ventricular ejection fraction. Echocardiography 2001 Nov;18(8):627-32.
48 Singal PK, Iliskovic N. Doxorubicin-induced cardiomyopathy. N Engl J Med 1998 Sep 24;339(13):900-5.
49 Meinardi MT, van Veldhuisen DJ, Gietema JA, Dolsma WV, Boomsma F, van den Berg MP, et al. Prospective evaluation of early cardiac damage induced by epirubicin-containing adjuvant chemotherapy and locoregional radiotherapy in breast cancer patients. J Clin Oncol 2001 May 15;19(10):2746-53.
50 Nielsen D, Jensen JB, Dombernowsky P, Munck O, Fogh J, Brynjolf I, et al. Epirubicin cardiotoxicity: a study of 135 patients with advanced breast cancer. J Clin Oncol 1990 Nov 1;8(11):1806-10.
51 Jensen BV, Skovsgaard T, Nielsen SL. Functional monitoring of anthracycline cardiotoxicity: a prospective, blinded, long-term observational study of outcome in 120 patients. Ann Oncol 2002 May;13(5):699-709.
52 Nousiainen T, Jantunen E, Vanninen E, Hartikainen J. Early decline in left ventricular ejection fraction predicts doxorubicin cardiotoxicity in lymphoma patients. Br J Cancer 2002 Jun 5;86(11):1697-700.
53 Steinherz LJ, Steinherz PG, Tan CT, Heller G, Murphy ML. Cardiac toxicity 4 to 20 years after completing anthracycline therapy. JAMA 1991 Sep 25;266(12):1672-7.
54 Belham M, Kruger A, Mepham S, Faganello G, Pritchard C. Monitoring left ventricular function in adults receiving anthracycline-containing chemotherapy. Eur J Heart Fail 2007 Apr;9(4):409-14.
55 Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. Circulation 2002 Mar 19;105(11):1387-93.
56 Bhatia RS, Tu JV, Lee DS, Austin PC, Fang J, Haouzi A, et al. Outcome of heart failure with preserved ejection fraction in a population-based study. N Engl J Med 2006 Jul 20;355(3):260-9.
57 Bu'Lock FA, Mott MG, Oakhill A, Martin RP. Left ventricular diastolic function after anthracycline chemotherapy in childhood: relation with systolic function, symptoms, and pathophysiology. Br Heart J 1995 Apr;73(4):340-50.
58 Schmitt K, Tulzer G, Merl M, Aichhorn G, Grillenberger A, Wiesinger G, et al. Early detection of doxorubicin and daunorubicin cardiotoxicity by echocardiography: diastolic versus systolic parameters. Eur J Pediatr 1995 Mar;154(3):201-4.
59 Cottin Y, Touzery C, Coudert B, Gilles A, Walker P, Massing JL, et al. Impairment of diastolic function during short-term anthracycline chemotherapy. Br Heart J 1995 Jan;73(1):61-4.
60 Leandro J, Dyck J, Poppe D, Shore R, Airhart C, Greenberg M, et al. Cardiac dysfunction late after cardiotoxic therapy for childhood cancer. Am J Cardiol 1994 Dec 1;74(11):1152-6.
61 Clements IP, Sinak LJ, Gibbons RJ, Brown ML, O'Connor MK. Determination of diastolic function by radionuclide ventriculography. Mayo Clin Proc 1990 Jul;65(7):1007-19.
62 Parmentier S, Melin JA, Piret L, Beckers C. Assessment of left ventricular diastolic function in patients receiving anthracycline therapy. Eur J Nucl Med 1988;13(11):563-7.
63 Santin JC, Deheinzelin D, Junior SP, Lopes LF, de Camargo B. Late echocardiography assessment of systolic and diastolic function of the left ventricle in pediatric cancer survivors after anthracycline therapy. J Pediatr Hematol Oncol 2007 Nov;29(11):761-5.
64 Farah CS, Reinach FC. The troponin complex and regulation of muscle contraction. FASEB J 1995 Jun 1;9(9):755-67.
65 Jaffe AS, Ravkilde J, Roberts R, Naslund U, Apple FS, Galvani M, et al. It's time for a change to a troponin standard. Circulation 2000 Sep 12;102(11):1216-20.
66 Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000 Sep;36(3):959-69.
67 Germanakis I, Anagnostatou N, Kalmanti M. Troponins and natriuretic peptides in the monitoring of anthracycline cardiotoxicity. Pediatr Blood Cancer 2008 Sep;51(3):327-33.
68 Daniels LB, Maisel AS. Natriuretic Peptides. Journal of the American College of Cardiology50(25):2357-68.
69 Hunt SA, Abraham WT, Chin MH, Feldman AM, Francis GS, Ganiats TG, et al. ACC/AHA 2005 Guideline Update for the Diagnosis and Management of Chronic Heart Failure in the Adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure): developed in collaboration with the American College of Chest Physicians and the International Society for Heart and Lung Transplantation: endorsed by the Heart Rhythm Society. Circulation 2005 Sep 20;112(12):e154-e235.
70 Del Ry S, Passino C, Emdin M, Giannessi D. C-type natriuretic peptide and heart failure. Pharmacological Research 2006 Nov;54(5):326-33.
71 Sandri MT, Salvatici M, Cardinale D, Zorzino L, Passerini R, Lentati P, et al. N-terminal pro-B-type natriuretic peptide after high-dose chemotherapy: a marker predictive of cardiac dysfunction? Clin Chem 2005 Aug;51(8):1405-10.
72 Wu AH. Serial testing of B-type natriuretic peptide and NTpro-BNP for monitoring therapy of heart failure: the role of biologic variation in the interpretation of results. Am Heart J 2006 Nov;152(5):828-34.
73 Schwartz CL, Hobbie WL, Truesdell S, Constine LC, Clark EB. Corrected QT interval prolongation in anthracycline-treated survivors of childhood cancer. J Clin Oncol 1993 Oct;11(10):1906-10.
74 Bender KS, Shematek JP, Leventhal BG, Kan JS. QT interval prolongation associated with anthracycline cardiotoxicity. J Pediatr 1984 Sep;105(3):442-4.
75 Iwata N, Karasawa M, Omine M, Maekawa T, Suzuki T, Kawai Y. Aclarubicin-associated QTC prolongation and ventricular fibrillation. Cancer Treat Rep 1984 Mar;68(3):527-9.
76 Meinardi MT, van Veldhuisen DJ, Gietema JA, Dolsma WV, Boomsma F, van den Berg MP, et al. Prospective evaluation of early cardiac damage induced by epirubicin-containing adjuvant chemotherapy and locoregional radiotherapy in breast cancer patients. J Clin Oncol 2001 May 15;19(10):2746-53.
77 Brooksby P, Batin PD, Nolan J, Lindsay SJ, Andrews R, Mullen M, et al. The relationship between QT intervals and mortality in ambulant patients with chronic heart failure. The united kingdom heart failure evaluation and assessment of risk trial (UK-HEART). Eur Heart J 1999 Sep;20(18):1335-41.
78 Bast A, Kaiserova H, den Hartog GJ, Haenen GR, van der Vijgh WJ. Protectors against doxorubicin-induced cardiotoxicity: flavonoids. Cell Biol Toxicol 2007 Jan;23(1):39-47.
79 Gupta M, Thaler HT, Friedman D, Steinherz L. Presence of prolonged dispersion of QT intervals in late survivors of childhood anthracycline therapy. Pediatr Hematol Oncol 2002 Dec;19(8):533-42.
80 Galetta F, Franzoni F, Cervetti G, Cecconi N, Carpi A, Petrini M, et al. Effect of epirubicin-based chemotherapy and dexrazoxane supplementation on QT dispersion in non-Hodgkin lymphoma patients. Biomed Pharmacother 2005 Dec;59(10):541-4.
81 Nakamae H, Tsumura K, Akahori M, Terada Y, Yamane T, Hayashi T, et al. QT dispersion correlates with systolic rather than diastolic parameters in patients receiving anthracycline treatment. Intern Med 2004 May;43(5):379-87.
82 de Bruyne MC, Hoes AW, Kors JA, Hofman A, van Bemmel JH, Grobbee DE. QTC Dispersion Predicts Cardiac Mortality in the Elderly : The Rotterdam Study. Circulation 1998 Feb 10;97(5):467-72.
83 Malik M, Batchvarov VN. Measurement, interpretation and clinical potential of QT dispersion. Journal of the American College of Cardiology 2000 Nov 15;36(6):1749-66.
84 Shah RR. Drug-induced QT dispersion: does it predict the risk of torsade de pointes? Journal of Electrocardiology 2005 Jan;38(1):10-8.

85 Postma A, Bink-Boelkens MT, Beaufort-Krol GC, Kengen RA, Elzenga NJ, Schasfoort-van Leeuwen MJ, et al. Late cardiotoxicity after treatment for a malignant bone tumor. Med Pediatr Oncol 1996 Apr;26(4):230-7.
86 Tjeerdsma G, Meinardi MT, van der Graaf WTA, van den Berg MP, Mulder NH, Crijns HJGM, et al. Early detection of anthracycline induced cardiotoxicity in asymptomatic patients with normal left ventricular systolic function: autonomic versus echocardiographic variables. Heart 1999 Apr 1;81(4):419-23.
87 Legha SS, Benjamin RS, Mackay B, Ewer M, Wallace S, Valdivieso M, et al. Reduction of doxorubicin cardiotoxicity by prolonged continuous intravenous infusion. Ann Intern Med 1982 Feb;96(2):133-9.
88 van Dalen EC, van der Pal HJ, Caron HN, Kremer LC. Different dosage schedules for reducing cardiotoxicity in cancer patients receiving anthracycline chemotherapy. Cochrane Database Syst Rev 2009;(4):CD005008.
89 Myers C, Bonow R, Palmeri S, Jenkins J, Corden B, Locker G, et al. A randomized controlled trial assessing the prevention of doxorubicin cardiomyopathy by N-acetylcysteine. Semin Oncol 1983 Mar;10(1 Suppl 1):53-5.
90 Iarussi D, Auricchio U, Agretto A, Murano A, Giuliano M, Casale F, et al. Protective effect of coenzyme Q10 on anthracyclines cardiotoxicity: control study in children with acute lymphoblastic leukemia and non-Hodgkin lymphoma. Mol Aspects Med 1994;15 Suppl:s207-s212.
91 Wagdi P, Rouvinez G, Fluri M, Aeschbacher B, Thoni A, Schefer H, et al. [Cardioprotection in chemo- and radiotherapy for malignant diseases--an echocardiographic pilot study]. Praxis (Bern 1994) 1995 Oct 24;84(43):1220-3.
92 Whittaker JA, Al Ismail SA. Effect of digoxin and vitamin E in preventing cardiac damage caused by doxorubicin in acute myeloid leukaemia. Br Med J (Clin Res Ed) 1984 Jan 28;288(6413):283-4.
93 Task FM, Swedberg K, Writing Committee:, Cleland J, Dargie H, Drexler H, et al. Guidelines for the diagnosis and treatment of chronic heart failure: executive summary (update 2005): The Task Force for the Diagnosis and Treatment of Chronic Heart Failure of the European Society of Cardiology. European Heart Journal 2005 Jun 1;26(11):1115-40.
94 Silber JH, Cnaan A, Clark BJ, Paridon SM, Chin AJ, Rychik J, et al. Enalapril to prevent cardiac function decline in long-term survivors of pediatric cancer exposed to anthracyclines. J Clin Oncol 2004 Mar 1;22(5):820-8.
95 Silber JH, Cnaan A, Clark BJ, Paridon SM, Chin AJ, Rychik J, et al. Design and baseline characteristics for the RNA Inhibitor After Anthracycline (AAA) study of cardiac dysfunction in long-term pediatric cancer survivors. Am Heart J 2001 Oct;142(4):577-85.
96 Hauser M, Wilson N. Anthracycline induced cardiomyopathy: successful treatment with angiotensin converting enzyme inhibitors. Eur J Pediatr 2000 May;159(5):389.
97 Jensen BV, Nielsen SL, Skovsgaard T. Treatment with angiotensin-converting-enzyme inhibitor for epirubicin-induced dilated cardiomyopathy. Lancet 1996 Feb 3;347(8997):297-9.
98 Toko H, Oka T, Zou Y, Sakamoto M, Mizukami M, Sano M, et al. Angiotensin II type 1a receptor mediates doxorubicin-induced cardiomyopathy. Hypertens Res 2002 Jul;25(4):597-603.
99 Iqbal M, Dubey K, Anwer T, Ashish A, Pillai KK. Protective effects of telmisartan against acute doxorubicin-induced cardiotoxicity in rats. Pharmacol Rep 2008 May;60(3):382-90.
100 Soga M, Kamal FA, Watanabe K, Ma M, Palaniyandi S, Prakash P, et al. Effects of angiotensin II receptor blocker (candesartan) in daunorubicin-induced cardiomyopathic rats. Int J Cardiol 2006 Jun 28;110(3):378-85.
101 Sacco G, Bigioni M, Evangelista S, Goso C, Manzini S, Maggi CA. Cardioprotective effects of zofenopril, a new angiotensin-converting enzyme inhibitor, on doxorubicin-induced cardiotoxicity in the rat. Eur J Pharmacol 2001 Feb 23;414(1):71-8.
102 Okumura K, Jin D, Takai S, Miyazaki M. Beneficial effects of angiotensin-converting enzyme inhibition in adriamycin-induced cardiomyopathy in hamsters. Jpn J Pharmacol 2002 Feb;88(2):183-8.
103 Tokudome T, Mizushige K, Noma T, Manabe K, Murakami K, Tsuji T, et al. Prevention of doxorubicin (adriamycin)-induced cardiomyopathy by simultaneous administration of angiotensin-converting enzyme inhibitor assessed by acoustic densitometry. J Cardiovasc Pharmacol 2000 Sep;36(3):361-8.
104 Cardinale D, Colombo A, Sandri MT, Lamantia G, Colombo N, Civelli M, et al. Prevention of High-Dose Chemotherapy-Induced Cardiotoxicity in High-Risk Patients by Angiotensin-Converting Enzyme Inhibition. Circulation 2006 Dec 5;114(23):2474-81.
105 Nakamae H, Tsumura K, Terada Y, Nakane T, Nakamae M, Ohta K, et al. Notable effects of angiotensin II receptor blocker, valsartan, on acute cardiotoxic changes after standard chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisolone. Cancer 2005 Dec 1;104(11):2492-8.
106 van Dalen EC, Caron HN, Dickinson HO, Kremer LC. Cardioprotective interventions for cancer patients receiving anthracyclines. Cochrane Database Syst Rev 2008;(2):CD003917.
107 Hasinoff BB. The interaction of the cardioprotective agent ICRF-187 [(+)-1,2-bis(3,5-dioxopiperazinyl-1-yL)propane); its hydrolysis product (ICRF-198); and other chelating agents with the Fe(III) and Cu(II) complexes of adriamycin. Agents Actions 1989 Mar;26(3-4):378-85.