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. Since then, numerous analogues have been developed, of which doxorubicin (Adriamycin®) is still the most commonly used (figure 1). Doxorubicin is used in the treatment of a wide range of malignancies, including breast cancer, ovarian cancer, leukemia and sarcomas.

Soon after the introduction of doxorubicin, cardiotoxic side-effects were noted. These effects are commonly divided into acute, sub-acute and chronic effects. 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/m² and 47% for doses over 700 mg/m². Other risk-factors include age and gender. Commonly used concomitant therapies in the treatment of cancers, like radiation and Herceptin, are also known to increase the occurrence of doxorubicin induced cardiotoxicity.

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. 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.

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’. 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 ($O_2^{-}$) (figure 2). These reactive oxygen species induce apoptosis, both via the extrinsic (Fas-mediated) and the intrinsic (mitochondrial) pathway. Cardiomyocytes are especially vulnerable to doxorubicin-induced apoptosis, as they contain low levels of ROS scavenging enzymes. 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.

Secondary alcohol metabolites

Doxorubicin may also cause cardiac myopathy by the formation of its secondary alcohol metabolites such as doxorubicinol (doxo). 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). 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 doxo 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$$^•$- + H$_2$O$_2$ → OH$^•$ + OH$^-$ + O$_2$). This reaction can only occur when an intracellular pool of free iron is present.(19) To date, it has not been completely elucidated how such a pool of free iron is formed within the cardiomyocyte. Most of the iron is stored in the iron-storage protein ferritin. Conflicting evidence exists with regard to iron release from ferritin in the presence of doxorubicin. Earlier studies showed that the presence of O$_2$$^•$- and the semiquinone of doxorubicin enabled Fe$^{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-IRE binding.(27) Further investigations revealed that low (sub-clinical) concentrations of doxorubicin actually increased IRP1-IRE 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-IRE binding by formation of disulfide complexes.(13) The influence of doxorubicin on IRP2-IRE binding is less extensively investigated. However, doxorubicin also appears to decrease IRP2-IRE binding.(29;30) It thus seems that doxorubicin favors iron sequestration over iron uptake by diminished IRP1- and IRP2-IRE 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$$^•$- + 2H$^+$ → H$_2$O$_2$) the reaction in which O$_2$$^•$- is converted to hydrogen peroxide, which is further degraded by catalase to water. (2H$_2$O$_2$ → 2 H$_2$O + 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 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-proBNP 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-prolongation, 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. 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.

### 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 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. In the treatment of anthracycline-induced cardiac failure, treatment with ACE-inhibitors was efficacious, too. 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. It appears that in clinical practice, too, ACE inhibitors and ATII-antagonists are beneficial in patients treated with anthracyclines. 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. This compound is capable to chelate intracellular iron (complexes), thereby preventing the formation of free radicals. 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.
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  Formation of ROS after anthracycline administration

Figure 3  Proposed mechanisms of iron-mediated cardiotoxicity

Physiological scenario with iron deficiency and iron overload

Proposed scenarios in the presence of doxorubicin

Increased IRP-1 binding

Blocked IRP-1 binding


Corapcioglu F, Sarper N, Berk F, Sahin T, Zengin E, Demir H. Evaluation of anthracycline-induced early left ventricular dysfunction in...
anthracycline-induced cardiotoxicity, a pathophysiology-based approach for early detection and protective strategies

CHAPTER 1
introduction and outline of the thesis


107 Hasinoff BB. The interaction of the cardioprotective agent IC81-187 (+)-1,2-bis(3,5-dioxopiperazinyl-1-yl)propane; its hydrolysis product (IC81-198); and other chelating agents with the Fe(III) and Cu(II) complexes of adriamycin. Anticancer Drugs 1998 Mar;9(4):378-85.