Figure 1  Mean left ventricular ejection fraction (a) and e/a-ratio (b) for pc-sod (open circles) and placebo (closed circles) and 95%-confidence intervals (pc-sod down, placebo up) at baseline and 1, 4 and 9 months post-chemotherapy.

Figure 2  Mean nT-proBNP (a) concentrations, ng/L, and qTc (b), milliseconds, linear corrected for heart rate according to Framingham, during chemotherapy and follow up for pc-sod (open circles) and placebo (closed circles) and 95%-confidence intervals (pc-sod up, placebo down) during the course and 1, 4, 9 months post-chemotherapy.

CHAPTER 8

Summary and Discussion
SUMMARY AND DISCUSSION

Traditionally drug development starts with the evaluation of kinetics and tolerability, while in a later stage efficacy is evaluated. An alternative approach that includes biomarkers for clinical endpoints early in the clinical development has been advocated, with potential gains in time and information content of the development process.\(^1;2\) This thesis described the such an approach for a drug to inhibit anthracycline-induced cardiotoxicity.

The thesis comprises two parts: in chapter 2, 3 and 4 we tried to further identify biomarkers suitable for detection of anthracycline-induced cardiotoxicity with an attempt to provide further insight in the pathophysiology of anthracycline-induced cardiotoxicity. In chapter 5, 6 and 7 the development of a novel compound shown to be effective against anthracycline-induced cardiotoxicity in animals, was described.

**Biomarkers for clinical endpoints in anthracycline-induced cardiotoxicity**

Although the underlying mechanisms are still not completely unravelled, reactive oxygen species (ROS), which are formed in the presence of non-protein bound iron (NPI), are likely to play a pivotal role in anthracycline-induced cardiotoxicity. These ROS lead to apoptosis of cardiomyocytes eventually causing cardiomyopathy and clinical heart failure. Several stages in the development of anthracycline-induced cardiac failure may be evaluated using biomarkers. Therefore a comprehensive set of biomarkers, including markers of oxidative stress, myocardial injury and remodelling, and markers related to the inflammatory processes that accompany the injury were selected. Theoretically, a combination of these biomarkers can be used to assess the risk for the future development of cardiac failure. Secondly a model using a combination of these markers could be useful in the evaluation of new protective compounds against anthracycline-induced cardiotoxicity.

**Oxidative stress** After anthracycline administration the damage to the myocardium begins with ROS. Therefore we included several parameters indicative of oxidative stress in our model. First oxidative damage was assessed by measuring the oxidation product of Low Density Lipoprotein (OxLDL). OxLDL is a predictor of mortality in congestive heart failure (CHF) and in a recent study it was shown that chronic exposure to OxLDL, as measured by antibodies against OxLDL, is associated with an increased morbidity and mortality in CHF.\(^3;4\) Other markers of oxidative stress used in our studies was the measurement of oxidative metabolites of bilirubin in urine. Urinary biopyrrins are associated with (intracellular) oxidative stress and conditions associated with free radical overload, including congestive heart failure and acute coronary syndromes and could therefore also be useful in the detection of anthracycline-induced oxidative stress.\(^5;6\)

In chapter 7 it was shown that OxLDL and urinary biopyrrins are not elevated after the administration of anthracyclines, suggesting that these markers are not suitable for the detection of anthracycline-induced cardiotoxicity in humans. This is in keeping with the knowledge that these markers were never directly linked to anthracycline-induced cardiotoxicity. The reason for this lack of response could be that these markers are not sensitive enough to detect oxidative stress caused by anthracyclines, or the dose used in our studies was too low to generate sufficient ROS (or a combination of both). Another possibility is that the effect of oxidative stress in vivo plays a less prominent role than previously thought.

In vitro and animal studies show that NPBI is an important factor in the generation of ROS.\(^7;9\) NPBI is elevated after administration of the free radical generating chemotherapeutic bleomycin.\(^10\) As discussed previously, NPBI has a pivotal role in the pathophysiology of anthracycline-induced cardiotoxicity.\(^11\) In this thesis (chapter 3) we further explored NPBI as a marker for oxidative stress after the administration of anthracyclines. It was shown that NPBI was increased for a short time after the administration of anthracyclines. These results give further insight in the in vivo mechanism of anthracycline-induced cardiotoxicity and indicate that after administration of anthracyclines NPBI indeed is involved...
in the mechanism of the cardiotoxicity of anthracyclines. The finding that NT-proBNP is released after the administration could also explain the efficacy of the iron chelator dexrazoxane against anthracycline-induced cardiotoxicity. Therefore our results and those of others indicate that NT-proBNP is an interesting marker to include in a pathophysiology-based model.

**INFLAMMATION** In the early stages of congestive heart failure pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), soluble ICAM and macrophage inhibiting protein (MIP) are elevated.\(^{(12)}\) It can be hypothesized that when the injury by anthracyclines is supposed to occur (during and shortly after the anthracycline infusions) these biomarkers will (transiently) increase as a sign of the myocardial damage.

In our study no changes in these biomarkers were observed during or shortly after chemotherapy. This can be attributed to several causes. First, because anthracyclines (or the dose that was used) simply do not induce the production of pro-inflammatory cytokines. Another possibility is that anthracyclines suppress the production of pro-inflammatory cytokines by a yet unknown mechanism or that these cytokines exert a local reaction and that this goes unnoticed when using systemic venous blood samples. Finally, it cannot be excluded that the concomitant administration of corticosteroids suppressed the inflammatory reaction after anthracycline administration. Inflammatory markers therefore are of limited use in a model for early evaluation of anthracycline-induced cardiotoxicity.

**CARDIAC INJURY** All the earlier described processes eventually lead to cardiac injury and failure. It is necessary, therefore, to include markers of cardiac function. Unfortunately, traditional markers of cardiac function, such as left ventricular ejection fraction (LVEF), measured by echocardiography of nuclear imaging, are unsuitable for the early detection of anthracycline-induced cardiotoxicity.\(^{(11)}\) This is merely because a decline in LVEF occurs in a stage of chronic pathology, when damage to the myocardium is irreversible. and compensatory mechanisms like remodeling are exhausted. Several biochemical markers, including natriuretic peptides and cardiac troponins, could be indicative of cardiac injury and have been suggested to detect anthracycline-induced cardiotoxicity in an early stage of pathology.\(^{(13-16)}\) In chapter 2 of this thesis we further explored some of these markers (cardiac troponins, NT-proBNP and CK-MB) and showed that the administration of anthracyclines give an almost immediate two- to threefold increase of NT-proBNP, while no effects for the other markers was seen. As elevated levels of NT-proBNP are associated with an increased myocardial wall stretch, our findings suggest that the administration of anthracyclines causes an immediate increase in myocardial wall stress. It can only be speculated what the mechanism is for the increase in wall stress, as it is possible that the increase in NT-proBNP is either caused by direct damage to the myocardium or represents the neurohormonal response to the damage. Whatever the mechanism is, our findings suggest that NT-proBNP is suitable for the course-to-course evaluation of anthracycline-induced cardiotoxicity. Furthermore, it can be hypothesized that assuming this marker is an indication of myocardial wall stress, preventing the rise in this marker could be an indication of a cardioprotective effect. Indeed, there is evidence that the concurrent administration of ACE inhibitors (which reduce afterload) can protect against anthracycline-induced cardiotoxicity.\(^{(17-18)}\)

In addition to biochemical markers, electrocardiographic parameters could also be indicative of cardiac failure. A marker that of interest is cardiac repolarization. Repolarization is represented in the ECG by the length of QT-interval. The QT-interval may be prolonged in the failing heart and studies related QT-prolongation to the occurrence of heart failure.\(^{(19)}\) Lengthening of the QT-interval has also been described after administration of anthracycline and has therefore been suggested as an early marker for anthracycline-induced cardiomyopathy.\(^{(16)}\) The research described in this thesis showed that anthracyclines directly affect repolarization, as a prolongation of the QT-interval occurred after administration of anthracyclines. In addition, the results in chapter 4 suggest that repolarization reserve, which represents lability of repolarization, is affected by the administration of anthracyclines. It was shown that the repolarization reserve, as measured by a new method to assess the beat-to-beat variation in QT-interval, increases after the administration of anthracyclines.
As anthracyclines are not known to block cardiac ion channels, it is possible that both effects (prolongation of the QT-interval and the increased lability of repolarization), is an early sign of the cardiotoxic effects of anthracyclines. These results point to a potentially powerful non-invasive technique to evaluate anthracycline-induced cardiotoxicity at an early stage.

In summary, in this thesis an extensive array of (bio)markers was evaluated for the early detection of anthracycline-induced cardiotoxicity. It can be concluded that particularly the markers of cardiac failure (e.g. NT-proBNP and prolongation of the QT-interval) could be suitable for the early detection of anthracycline-induced cardiotoxicity.

The multi-marker approach described in this thesis could be a powerful tool in the development of cardioprotective compounds. This is in keeping with a research study that used a multi-marker approach to predict heart failure in the general community.(20)

**Protective Strategies**

The second part of this thesis consisted of the development of a new compound that could protect against anthracycline-induced cardiotoxicity.

The general belief was that the toxicity of anthracyclines could almost solely be explained by the effect of free radicals, which are formed intracellularly in the presence of iron. Therefore, numerous free radical scavengers have been evaluated in the past decade, both preclinically and clinically, to antagonize the toxic effects of anthracyclines – so far with limited effect, however.(21) As a possible explanation for this failure it has been suggested that the exogenously administered antioxidants did not reach the cytosol, or had too short a half-life. There is also evidence that the therapeutic range of antioxidants is narrow.(22;23) One of the most important antioxidants is superoxide dismutase (SOD), which is present in the cytosol, mitochondria and (in small amounts) extracellularly. SOD has an important function in scavenging free radicals formed in the presence of anthracyclines.

To overcome some of the disadvantages of SOD a lecithinized superoxide dismutase (PC-SOD) was developed which has a prolonged half-life and enhanced affinity for the cell membrane. (22;23) Indeed, studies in animals showed that PC-SOD could be efficacious against anthracycline-induced cardiotoxicity. In chapter 5 and 6 of the thesis the first-in-human studies of this compound are described. It was shown that in humans, too, PC-SOD had a prolonged half-life and an increased SOD activity until 24 hours after administration. It can be hypothesized that these characteristics make PC-SOD a possible candidate to be a protective agent in anthracycline-induced cardiotoxicity. Using the model described earlier, the efficacy of PC-SOD was evaluated in female breast-cancer patients, who received doxorubicin as adjuvant treatment. PC-SOD failed to show efficacy. Among the possible causes for this failure discussed in chapter 7, the most likely explanation is that free radical formation is not the sole explanation for the cardiotoxicity of anthracyclines. Evidence for this can be found in the fact that the anti-cardiotoxic effect of dexrazoxane is mediated by its ability to specifically inhibit doxorubicin-induced DNA damage in cardiomyocytes and probably not by counteracting the formation of free radicals.(24) Secondly, mitoxantrone, which is not known to cause free radical overload, also causes cardiotoxicity.(25-27) The findings in this thesis support the increasing knowledge that ROS are not solely responsible for anthracycline-induced cardiotoxicity and further preclinical research is necessary to elucidate the exact mechanism of anthracycline-induced cardiotoxicity.

**Future directives**

**Potentially new biomarkers** In this thesis a comprehensive set of biomarkers is used, recently two new biomarkers have been discovered, ST2 and galectin-3.(12;28) Both markers are associated with cardiac fibrosis and remodeling and can be used to detect heart failure in an early stage.(12;28) There is a case to evaluate these markers in anthracycline-induced cardiotoxicity as it can be hypothesized that administration of anthracyclines leads to cardiac remodeling and fibrosis. The latter is supported by the finding that late gadolinium enhancement, which is a marker of cardiac fibrosis, can be detected with cardiac MRI after
New chemotherapeutic agents like liposomal anthracyclines may solve the problem entirely. A recent meta-analysis demonstrated that the occurrence of clinical and subclinical cardiotoxicity was considerably reduced with these agents when compared to doxorubicin. (34)

In addition, some other less ordinary strategies, such as modifying intracellular transcription factors, are worth exploring. The observation that concurrent treatment with the monoclonal antibody against the HER2/neu oncogene initially augments the occurrence of heart failure, leads to the hypothesis that upregulating HER2/neu could protect against anthracycline-induced cardiotoxicity. Indeed there is preclinical evidence that this protects cardiomyocytes from toxicity of anthracyclines. (35-37) Another interesting target is GATA4, a transcription factor that regulates myocyte differentiation and sarcomere synthesis, and influences survival and several cardiac genes that play a role in anti-apoptotic signaling. (38;39). Additionally it suppresses anthracycline-induced apoptosis. (40) As GATA4 overexpression by alpha-adrenergic agonists antagonizes anthracycline-induced cardiotoxicity it has been suggested that administration of alpha-adrenergic agonists could be cardioprotective in anthracycline induced heart failure. This seems paradoxical as increasing adrenergic drive has a deleterious influence on the outcome in heart failure. Nevertheless some authors suggest that the use of alpha-adrenergic agonists should be evaluated. (41) Finally, it has been suggested that exercise could prevent against anthracycline-induced cardiotoxicity by increasing neuregulin/erbB signaling. (41) It can be expected that when the exact mechanism of anthracycline-induced cardiotoxicity is further elucidated even more protective options will emerge.

**Overall conclusion**

In this thesis the development of a pathophysiology-based method for the early evaluation of anthracycline-induced cardiotoxicity was described. We evaluated a comprehensive array of biomarkers, representing several aspects of anthracycline-induced cardiotoxicity, including cardiac injury and remodeling, free
radical overload and the inflammation accompanying the injury. It was shown that predominantly the markers of cardiac injury may be suitable for the early detection of anthracycline-induced cardiotoxicity. In the second part of this thesis we evaluated a new, free-radical scavenging compound against anthracycline-induced cardiotoxicity using this approach. The failure of this compound to show efficacy against anthracycline-induced cardiotoxicity in our model suggests that a broader approach toward the mechanism of anthracycline-induced cardiotoxicity is necessary.


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