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

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CHAPTER 7

Evaluation of lecithinized human recombinant super oxide dismutase as cardioprotectant in anthracycline-treated breast cancer patients

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ABSTRACT

AIM Anthracycline-induced cardiotoxicity is (partly) mediated by free radicals overload. A randomized study was performed in breast cancer patients to investigate whether free-radical scavenger Super Oxide Dismutase (SOD) protects against anthracycline-induced cardiotoxicity as measured by changes in echo- and electrocardiography and an array of biomarkers.

METHODS AND RESULTS Eighty female, chemotherapy-naïve breast cancer patients (median age 49, range 24-67) scheduled for 4 or 5 courses of adjuvant three-weekly doxorubicin plus cyclophosphamide (AC) chemotherapy, were randomly assigned to receive 80 mg PC-SOD (human recombinant SOD bound to lecithin) or placebo, administered intravenously (IV) immediately prior to each AC course.

The primary end point was protection against cardiac damage evaluated using echocardiography, QT-assessments, and a set of biochemical markers for myocardial function, oxidative stress and inflammation. Assessments were performed before and during each course of chemotherapy, and at 1, 4 and 9 months after completion of chemotherapy regimen. In all patients cardiac effects such as increases in NT-proBNP concentration and prolongation of the QTc-interval were noticed. There were no differences between the PC-SOD and placebo-treated patients in systolic or diastolic cardiac function or for any other of the biomarkers used to assess cardiac effects of anthracyclines.

CONCLUSION PC-SOD at a dose of 80 mg IV is not cardioprotective in patients with breast carcinoma treated with anthracyclines.

CLINICAL TRIAL REGISTRATION INFORMATION The study is registered at www.controlled-trials.com, number ISRCTN56637853.

INTRODUCTION

Anthracyclines are widely used in treatment regimens of cancer, including breast cancer. Their use is hampered by occurrence of irreversible cardiotoxicity which typically manifests as congestive heart failure (CHF) months to years after anthracycline exposure. It is primarily related to cumulative anthracycline dose and it seems that females are affected more often than males.(1;2) The incidence increases from 5% in patients receiving doses up to 400 mg/m2 to 48% in patients receiving more then 700 mg/m2 of doxorubicin.(1) Although less toxic analogues such as epi-doxorubicin have been developed, anthracycline-induced cardiotoxicity remains a clinical problem.(3) As the decline in ejection fraction and clinically manifest CHF usually become apparent relatively late after anthracycline therapy, it is difficult to assess the cardiotoxic effects of anthracyclines early. However, anthracycline cardiac toxicity has also been reported to occur after only single dose administration.(1) This suggests that it may be possible to use (bio)markers of cardiac effects due to anthracyclines occurring early and that may be predictive of the late toxicity. Indeed, several markers such as QT-prolongation and changes in NT-proBNP and cardiac troponin levels have been suggested to be such early markers.(4-7) These markers can potentially also be used to assess the effects of putative protective strategies.(4)

The mechanism of anthracycline-induced cardiotoxicity has not been fully elucidated, but formation of reactive oxidative species (ROS), such as the superoxide (O_2-) radical seem to play a major role.(8) Superoxide dismutase (SOD) is an important scavenger of these ROS and its use to prevent organ damage mediated by free radical overload has been investigated.(8) However, the currently existing therapies using exogenous SOD as a protectant has been limited by for instance its short half-live and low affinity for the cell membrane.(9) Lecithinized SOD (PC-SOD) has a 100-200 fold higher affinity for the cell membrane and improved free radical scavenging properties.(10) Several animal-models, including a rodent doxorubicin-induced cardiotoxicity model, showed that PC-SOD protected against free-radical mediated injuries.(11-20)

Early clinical studies in healthy subjects showed that a single intravenous (IV) dose of 80 mg PC-SOD resulted in increased SOD-activity *in vivo* for 16-24 hours.(21;22)

The efficacy of PC-SOD as cardioprotective agent against anthracycline-induced cardiotoxicity was explored in an early phase II study in woman with breast cancer, using serial echocardiography measurements, electrocardiography and a set of (bio)markers, reflecting myocardial function, oxidative stress and inflammation.(4;7;23-28)

METHODS

Patient population

This multi-center, randomized, placebo-controlled trial was performed in female patients with early-stage breast cancer eligible for adjuvant doxorubicin and cyclophosphamide (AC) chemotherapy. Patients were scheduled to receive either 4 or 5 AC cycles according to national guidelines at that time. Prior or concomitant use of cardiotoxic medication was an exclusion criterion. Patients with distant metastases, a history of other malignant disease, a life expectancy of less than one year, pre-existing cardiovascular diseases, elevated transaminases above 3 times the upper limit of normal and patients of whom we were unable to obtain a good quality echocardiogram before study drug administration were excluded.

The institutional review board of Leiden University Medical Center (LUMC) approved the study protocol. All patients gave written informed consent before participation and the study was conducted in accordance with the declaration of Helsinki (South Africa 1996 amendment), Good Clinical Practice and all applicable local laws and regulations.

Study protocol

This study was coordinated and designed by the Centre of Human Drug Research (CHDR) and carried out in 5 oncology centers in The Netherlands. After randomization (1:1 to 80mg PC-SOD or placebo) of eligible patients baseline assessments were done and the patients started their scheduled chemotherapy (4 or 5 courses) consisting of a combination of doxorubicin (60 mg/m2 over approximately 30 min) and cyclophosphamide (600 mg/m2 over approximately 30 min) administered IV. Patients were admitted to the hospital in the morning of each chemotherapy course. After baseline assessments were completed the patients received PC-SOD or placebo as a 1-hr IV infusion. Immediately thereafter, anti-emetics followed by AC. After discharge in the afternoon, a 24 hour visit took place in the morning of the following day. A similar procedure was repeated during a maximum of four courses. Patients receiving 5 courses received the study drug at the third course but no measurements were done. Median volume loading during the courses was 300ml per hour (in total approximately 850 ml in 4 hours). After completion of chemotherapy, follow up visits took place at 1, 4 and 9 months.

Study medication

PC-SOD consists of an average of 4 molecules lecithin derivative covalently bound to the human derived CuZn-SOD, produced by genetic recombination using *E.coli* as a host cell.(10) The lecithinized product has 3x103 U SOD-activity per mg. A single batch of the lyophilized formulation was used. The PC-SOD formulation consisted of 80 mg PC-SOD and 133mg sucrose, the placebo formulation only consisted of sucrose. PC-SOD and placebo were prepared for use by dissolution in 5% mannitol diluted with distilled water; all study medication was prepared at the LUMC hospital pharmacy.

Outcome measures

EFFICACY Efficacy assessments included echocardiography (left ventricular ejection fraction [LVEF], E/A ratio), electrocardiography [ECG] (QT-assessments) and blood sampling for biomarkers of cardiac function or damage (NT-proBNP, CK-MB and troponin T),

inflammation (macrophage inhibiting protein 1 [MIP-1], high sensitivity C-reactive protein [hsCRP], tumor necrosis factor alfa [TNF-a] and soluble intercellular adhesion molecule-1 [sICAM]), and oxidative stress (oxLDL, urinary biopyrrin and non-protein bound iron [NPBI]).

Echocardiography, ECGs and blood sampling for determination of NT-proBNP, CK-MB (mass) and troponin T concentrations were done at baseline and 1 (including NPBI), 4 and 9 months after a full chemotherapy regimen.

ECG and blood sampling (for all biomarkers) was done before and at 24 hours after the start of each chemotherapy course. In addition ECG-recordings were made and blood was sampled for determination of CK-MB (mass), troponin T and TNF-α concentration at 4 hours after the start of each chemotherapy course.

SAFETY During the study period hematology and blood chemistry were frequently assessed. Glomerular filtration rate (GFR) was determined during the courses from 24 hours creatinine clearance, during the follow up visits the MDRD formula was used.(29) In addition, at the first follow-up visit antibodies against PC-SOD were determined.

All (serious) adverse events ((S)AE) were monitored from inclusion until last follow up and (S)AE's and concomitant medication were classified according to the World Health Organization Adverse Reaction Terminology and drug (WHOart and WHOdrug) classification system.

After completion of the last chemotherapy cycle for every 10th patient (until 60 patients were included), an interim safety report was reviewed by an independent Data Monitoring Committee (DMC). This report included all occurred (S)AE and laboratory safety data. After each report the DMC informed the principal investigator if in their opinion the data raised any safety concerns. The DMC was blinded during the whole study period, but could request emergency deblinding of (a part of) the data when deemed necessary.

QUALITY OF LIFE Quality of life (QoL) was assessed using two validated questionnaires developed by the European Organization for Research and Treatment of Cancer (EORTC): QoL (EORTC QLQ-C30, version 3) and QoL (EORTC QLQ-BR23).(30) Qol was assessed at baseline, during each course and 1, 4 and 9 months after completion of chemotherapy.

PHARMACOKINETICS Blood was sampled for the determination of PC-SOD serum concentrations during the first and last course at baseline and directly, 4 hours and 23 hours after the end of the infusion of PC-SOD or placebo.

ECHOCARDIOGRAPHY Echocardiography was performed at two locations in the Netherlands: the department of cardiology of LUMC, Leiden and the department of cardiology of Maasstad Ziekenhuis, Rotterdam. The examinations were performed by a single echographer in each center and all examinations were supervised by an experienced cardiologist. To exclude inter-observer variability all echo assessments for each individual patient were done at one center.

The investigations consisted of routine imaging, M-mode imaging for measurement of left ventricular end-diastolic and end-systolic wall thickness (septum, posterior wall), fractional shortening and left ventricular ejection fraction (LVEF, calculated according to Teichholz).(31) Measurements were made from the parasternal long-axis (or short-axis) view. The ratio of early rapid ventricular filling over atrial assisted filling (E/A ratio) was measured using pulsed-wave Doppler. Regional systolic function was evaluated with visual assessment of wall motion (and wall motion score index, WMSI) according to the 16-segment model. The examinations were performed using AGEvivid-7 echocardiograph equipped with pulsed-wave Doppler in the LUMC and using a Hewlett-Packard HP 5500 with a S3 probe in the Maasstad Ziekenhuis.

ECG RECORDINGS AND ANALYSIS For each patient 5-minute ECG recording were made using the CardioPerfect device (Welch Allyn, Delft, The Netherlands). ECG recordings were analyzed after fiducial segment averaging (FSA) to obtain heart rate, and QT-interval. This analysis was done using Intraval (Advanced Medical Systems, Maasdam, the Netherlands).(20)

For the analyses correction of the QT-interval for heart rate was done using Bazett's formula (QTcB= QT/√ (RR)), Fredericia's cubic root QTcF=QT * (1/RR)[13];and using the linear correction method according to Framingham Heart Study (QTcL= QT + 0.154 * (1 – RR)).

Assays

Samples were assayed for NT-proBNP, troponin T and CK-MB (mass) and NPBI at the Central Clinical Chemical laboratory (CKCL) of LUMC. Lower limits of detection (inter- and intra-assay variability) were 5 ng/L (< 5.8%), 0.1 ng/mL (< 2.5%), 0.01 µg/mL (< 5.6%) and 0.01 µmol/L (< 9.2) for NT-proBNP, cTnT, CK-MB (mass) and NPBI respectively. Assays for TNF-a, hsCRP, sICAM, MIP-1a, oxLDL and urinary bioppyrin were performed at the Netherlands Organization for Applied Scientific Research (TNO). The lower limits of detection of the assays (inter- and intra-assay variability) were 0.12 pg/mL (<12.5%), 0.1 µg/L (< 10%), 0.35 ng/mL (< 12.5%), 10 pg/mL (< 10%), 1 mU/L (< 7.5%) and 0.1 U/L (< 12.5%) for TNFa, hsCRP, sICAM, MIP-1a, oxLDL and urinary biopyrrin respectively.

Serum PC-SOD concentrations were measured using an enzyme linked immunosorbent assay (ELISA), consisting of an antibody against human Cu, Zn-SOD, and a second antibody against human Cu, Zn-SOD conjugated with horseradish peroxidase. The assay has a lower limit of quantification 626 ng/mL and the coefficients of variation did not exceed 7.9% which was observed for the lower concentrations. Antibody formation against PC-SOD was measured by quantification of specific IgE, IgG and IgM titres as described previously.(21)

Statistical analyses

POWER As both the incidence of sub-clinical cardiotoxicity and the treatment effects were unknown, the power calculation of the study has been performed using a number of assumptions: (1) The incidence of subclinical cardiomyopathy in patients is 33%; (2) Animal experiments suggest that PC-SOD treatment prevents cardiomyopathy in 100% of the cases. It has therefore been estimated that the protection by PC-SOD will reduce incidence of cardiomyopathy from 33% to 5.5% in the patients. In order to be able to demonstrate this treatment effect (power=80%; 2-sided test; $p<0.05$) a total of 72 patients is required. Second an exploratory power calculation on the biomarkers has been performed. These showed that this study has 80% power to detect (2 sided test; $p<0.05$) a difference in NT-proBNP levels between the groups of approximately 53%.

EFFICACY AND SAFETY POPULATION Eighty female breast cancer patients were randomized to PC-SOD or placebo. After randomization one patient was excluded because of an abnormal echography at baseline. During the trial 7 patients were replaced: 4 patients dropped out (2 patient's request, 1 because of discontinuation of chemotherapy due to extreme nausea), 4 patients had incomplete echocardiographic assessments (2 due to logistic problems, 2 due to equipment failure). Data of replaced patients are used in both safety and efficacy analyses. Two patients were excluded from the efficacy analyses because of anomalies in PK-results, hence the safety population and efficacy population consisted of 79 and 77 patients respectively.

TREATMENT EFFECTS First, for each course the difference between the 24 hour and the baseline measurement was calculated and this series of four differences was compared between placebo and PC-SOD treatment. This short term effect was analyzed using a mixed model analysis of variance (SAS proc mixed) with visit as repeated factor within patient, treatment (PC-SOD/placebo), group (4 or 5 courses) and treatment by group, treatment by time, group by time and treatment by group by time as fixed effects. For ECG parameters, TNFa and CK-MB (mass) the difference between the measurement at 4 hour and baseline at the occasion was analyzed the same way.

Second, the baseline measurement of each course except the first and the follow-up measurements were compared between Placebo and PC-SOD. The long-term treatment effect for the echocardiographic, ECG parameters and CK-MB and NT-proBNP was analyzed using a mixed model analysis of variance (SAS proc mixed) with visit (occasion) as repeated factor within patient, treatment,

group, treatment by time, treatment by group, time by group and treatment by time by group as fixed effects. The baseline value of the first course was included as covariate.

TIME EFFECTS To assess the 4 and 24 hour difference from baseline within a course, the estimated differences from baseline were compared to 0 (no difference from baseline) within the first treatment mixed model for the ECG and biomarker parameters. The estimated difference between the course 1 baseline and follow up measurements (long-term time-effects) for the echocardiographic, ECG parameters, CK-MB and NT-proBNP were compared to 0 (no difference from baseline) within the second treatment mixed model.

PHARMACOKINETICS Compartmental pharmacokinetic analysis was performed using NONMEM Version VI software (GloboMax LLC, Hanover, MD), maximum serum concentration and half-life were reported.

ADDITIONAL (SUB-GROUP ANALYSES) All data was also analyzed excluding patients who received trastuzumab or left-sided radiotherapy as concomitant therapy.

All statistical analyses were performed using SAS for windows V9.1.2 (SAS Institute, Inc., Cary, NC, USA). The study is registered at www.controlled-trials.com, number ISRCTN56637853.

RESULTS

Baseline characteristics

The median age of the 79 patients who received at least one dose of PC-SOD and AC chemotherapy was 49 years (range 24-67 years). The median (min-max) number of courses was 4 (1 to 5) and 4 (2 to 5) for placebo and PC-SOD, combined with AC chemotherapy, respectively (table 1).

Safety

There were no clinically relevant findings related to PC-SOD treatment on clinical laboratory measurements, vital signs or ECG findings. GFR was stable during the study period. The AE pattern did not differ among treatment groups (table 2) and the majority of AE's could be attributed to the chemotherapeutics and were mild to moderate in intensity. Antibodies against PC-SOD were not detected in any of the patients

EFFICACY

Long-term effects

ECHOCARDIOGRAPHY LVEF (± SD) and E/A ratio (± SD) were 67% ± 6, 1.06 ± 0.28 and 64% ± 7, 1.1 ± 0.3 at baseline, in patients receiving placebo and PC-SOD respectively and the overall decline (95% confidence interval between brackets) was -1% (-2 to1%), 0.0 (0.0 to 0.0%) and -2% (-3 to -0%), 0.0 (0.0 to 0.0%) during the study (figure 1, table 3).

Differences (95%-confidence interval between brackets) between PC-SOD and placebo on LVEF and E/A ratio were -1% (-3 to 1%) and 0.0 (-0.1 to 0.0%) respectively.

WMSI did not change significantly during the trial and no differences between treatments were observed.

BIOMARKERS - MYOCARDIAL INJURY During courses and follow up the overall change (percentage change, 95%-confidence interval between brackets) of NT-proBNP and CK-MB was 32.0% (12.8 to 54.5), -5.7% (-12.4 to 1.4%) and 14.2% (-2.6 to 33.8%), -7.6% (-14.2 to -0.5%) in patients receiving placebo and PC-SOD respectively (figure 2, table 3).

The differences between PC-SOD and placebo were -13.5% (-30.9 to 8.2%) and -2.0% (-11.7 to 8.8%) for NT-proBNP and CK-MB respectively.

During the follow-up period in 10 patients (6 PC-SOD, 4 placebo) detectable (although not pathologically elevated) troponin levels were present.

ELECTROCARDIOGRAPHY Heart rate and QTc-interval (corrected using a linear method) increased during courses and follow up with 0.6 BPM (-1.5 to 2.8 BPM), 5 msec (3.8 to 11.3msec) and 4.0 BPM (1.9 to 6.2 BPM), 10.8 msec (7.0 to 14.7 msec) in patients with placebo or PC-SOD respectively (figure 3, table 3).

The differences between PC-SOD and placebo for heart rate, QT-interval, corrected QT-interval (Bazett) and corrected QT-interval (linear) were 3.4 BPM (0.4 to 6.5 BPM), -3.1 msec (-11.6 to 5.4 msec), 7.4 msec (1.9 to 12.9 msec) and 3.3 msec (-2.1 to 8.7 msec) respectively.

Effects within the courses

OXIDATIVE STRESS Urinary biopyrrin increased (percentage change, 95%-confidence interval between brackets) within the courses in the placebo group only, although this effect was not present for each individual course. Change at 24 hours was 13.0% (0.8 to 26.7%) and 3.4% (-7.9 to 16.0%) in patients receiving placebo and PC-SOD respectively. While OXLDL and NPBI levels did not change significantly between baseline and at 24 hours, the difference in percentage change (95%-confidence interval between brackets) between PC-SOD and placebo was 10.3% (-20.5 to 52.9%), 6.2% (0.2 to 12.5%) and -8.5% (-22.2 to 7.6%) for urinary biopyrrin, OXLDL and NPBI respectively (table 4).

MYOCARDIAL INJURY The overall increment at 24 hours post-dose for NT-PROBNP and CK-MB (mass) was 199.8% (154.6 to 253.0%), 8.2% (0.6 to 16.4%) and 263.8% (207.9 to 329.7%), 10.3% (2.5 to 18.8%) in patients receiving placebo and PC-SOD respectively.

The difference at 24 hours post-dose between PC-SOD and placebo was 21.4% (-3.9 to 53.3%) and 2.0% (-8.1 to 13.1%) for NT-PROBNP and CK-MB respectively (table 4).

INFLAMMATION Within the courses HSCRP and SLCAM did not change markedly, while TNF-α and MIP-1α declined with -23.2% (-30.5 to -15.1%), -46.9% (-64.5 to -20.4%) and -27.2% (-34.5 to -19.1%), -48.6% (-65.9 to -22.5%) in patients receiving placebo or PC-SOD respectively. For TNF-alpha this effect was already present at 4 hours post-dose. At 24 hours the difference between PC-SOD and placebo for HSCRP, SLCAM-1, TNF-α, MIP-1α was -2.9% (-19.0 to16.5%), -0.7% (-2.7 to 1.4%), -5.3% (-18.1 to 9.6%) and -3.3% (-45.7 to 72.1%) respectively (table 4).

ELECTROCARDIOGRAPHY Heart rate showed a small increment at 24 hour during the courses in the PC-SOD arm, changes were -1.2 BPM (-3.2 to 0.7 BPM) and 3.6 BPM (1.8 to 5.5 BPM) in patients receiving placebo and PC-SOD respectively. After each course the (corrected) QT-interval prolonged at 4 hours post-dose and increased further at 24 hours post-dose. Overall prolongation of the QTc interval (using a linear correction method) at 24 hours post-dose was 12.4 msec (8.8 to 15.9 msec) and 9.8 msec (6.4 to 13.2 msec) in patients receiving placebo and PC-SOD respectively.

The difference between PC-SOD and placebo at 24 hours after each chemotherapy cycle in heart rate, QT-interval, corrected QT-interval (Bazett), QT-interval (linear) was 4.9 BPM (2.2 to 7.6 BPM), -11.0 msec (-18.2 to -3.9 msec), 3.0 msec (-2.5 to 8.4 msec) and -2.7 msec (-7.4 to 1.9 msec) respectively (table 4).

Number of courses and other adjuvant therapy

It was also analyzed if other (potentially cardiotoxic) adjuvant therapy or the number courses influenced our results. As all analyses showed comparable results; only the full dataset was reported.

QUALITY OF LIFE In both treatment groups similar effects (decline) on QoL during the chemotherapy were observed (data not presented).

PHARMACOKINETICS Maximum serum concentrations were reached within 1 hour and amounted to 32.4 mg/L (SD 11.9) and 31.4 mg/L (SD 12.1) for the first and last visit respectively. The estimated half-life was approximately 20 hours.

PC-SOD did not show a protective effect on cardiotoxicity, as evidenced by differences in NT-proBNP concentration and prolongation of the QTc-interval, which occurred in all breast cancer patients undergoing AC chemotherapy. Also, echocardiographic systolic (LVEF) function or any of the array of the other biomarkers assessed did not show a clinical significant change during or following chemotherapy and were also not affected by PC-SOD.

Safety analyses did not show any unfavorable effects of PC-SOD at the administered dose, as laboratory assessments and AE patterns were similar between treatments.

The lack of a cardioprotective effect of PC-SOD at a dose of 80 mg IV on any of the markers of anthracycline-induced cardiotoxicity in chemo-naïve breast cancer patients may be explained by a lack of efficacy of PC-SOD at the dose used.

However, the negative findings in this study are in keeping with the results of several other studies, showing that exogenously administered free radical scavengers are not able to protect against anthracycline-induced cardiotoxicity and add to the increasing knowledge that free radical mediated injury is only partly involved in the pathogenesis of the cardiotoxicity of anthracyclines.(32-34) In addition, we were not able to demonstrate the occurrence of oxidative stress *in vivo*, as none of the biomarkers for oxidative stress changed after doxorubicin infusion.

Another reason for the lack of effects of PC-SOD (and maybe of free radical scavenging agents in general) could be that the therapeutic window of these agents seem to be narrow. This involves the observation that in animals a bell-shaped dose-response curve (higher doses of SOD showed less protection) is present after administration of (PC-) SOD.(11;35-39) If such a bell-shaped curve is also present in humans, this could implicate that in this study not the correct dosage was used. Although several mechanisms could be responsible for this bell-shaped effect curve, the most plausible explanation is (PC-) SOD causing excess ROS formation. In particular this concerns formation of H_2O_2 which has been shown to be capable to induce apoptosis in cardiomyocytes.(8;37;40-43)

Independent of the explanation of the failure of free radical scavenging agents as protective agents against anthracycline-induced cardiotoxicity, our study re-emphasizes the necessity to identify other strategies to reduce the risk of anthracycline-induced CHF.

We considered the possibility that the administered doses of anthracyclines did not induce sufficient myocardial damage to detect any prophylactic effect of PC-SOD, as LVEF and E/A ratio did not change markedly. However, the profound changes in NT-proBNP concentration and (corrected) QT-interval, indicate that in all patients indeed experienced some (subclinical) cardiotoxicity, as both markers are associated with the occurrence of anthracycline induced cardiac failure and an adverse outcome.(44)

A limitation of our study is that although the echo- and electrocardiographic and biochemical endpoints used in this study are well established markers of (anthracycline induced) cardiac damage and functional impairment, oxidative stress and inflammation, the study was not designed to detect differences in cardiac mortality or the occurrence of clinical CHF. Furthermore, some patients received additional potentially cardiotoxic treatments such as trastuzumab and/or radiotherapy. However, re-analysis of the data excluding these patients did not result in different findings. An additional yield of this trial is that we have demonstrated several robust biomarkers that are mechanistically associated with the Adriamycin-induced acute myocardial damage. These biomarkers could be used in future studies with other investigative agents that protect against this damaging effect.

In conclusion, we showed that IV administration of 80mg PC-SOD prior to each chemotherapy course was not efficacious as protective agent against anthracycline-induced cardiotoxicity, as evaluated by echocardiography, electrocardiography and a comprehensive array of biomarkers of myocardial damage, inflammation and oxidative stress, in female breast cancer patients treated with a combination of cyclophosphamide and doxorubicin for early stage breast cancer.

ROLE OF THE FUNDING SOURCE The funding source was involved in the design of the study. Data collection and analyses were performed by CHDR. All authors had access to all study data and had final responsibility for the decision to submit for publication.

CONFLICT OF INTEREST This study was financially supported by LTT-Bio-Pharma, Tokyo, Japan. J. Suzuki and Y. Mizushima were employees of LTT-Bio-Pharma.

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Table 1 Baseline Demographics and Clinical Characteristics

		Placebo (n = 40)	PC-SOD (n = 39)
Age, BMI and cumulative doxorubicin dose; median (range)			
Age (years)		47.0 (30 – 66)	50.0 (24 – 67)
BMI (kg/m2)		24.0 (19.4 – 37.7)	25.5 (19.6 – 38.5)
Cumulative doxorubicin dose (mg/m2)		240 (60 – 300)	240 (120 – 300)
Echocardiography; mean (SD)			
LVEF (%)		66 ± 6	64 ± 7
E/A ratio		1.06 ± 0.28	1.08 ± 0.28
Hemoglobin, renal function, and cardiac (bio)markers; mean (SD)			
Hemoglobin (mmol/L)		7.7 ± 0.7	8.0 ± 0.7
GFR (ml/min)		100.5 ± 23.9	106.3 ± 37.4
NT-proBNP (ng/L)		71 ± 46	84 ± 92
CK-MB mass (mg/L)		1.7 ± 0.9	1.7 ± 0.5
QTcL (msec)		426 ± 12.7	433 ± 24.0
Number of courses†; n (%)			
1		2 (3%)	0 (0%)
2		0 (0%)	1 (1%)
3		2 (3%)	0 (0%)
4		17 (22%)	20 (25%)
5		19 (24%)	18 (23%)
Adjuvant therapy; n (%)			
Hormonal therapy		24 (60%)	26 (67%)
Trastuzumab		0 (0%)	5 (13%)
Docetaxel		0 (0%)	4 (10%)
Gosereline		2 (5%)	3 (8%)
Radiotherapy; n (%)			
Right	Prior to chemotherapy	3 (8%)	3 (8%)
	After cessation of chemotherapy	3 (10%)	9 (23%)
Left	Prior to chemotherapy	4 (10%)	4 (10%)
	After cessation of chemotherapy	6 (15%)	9 (22%)

† Number of courses doxorubicin, cyclophosphamide (all patients received PC-SOD or placebo prior to their chemotherapy courses). No statistical comparison was done at baseline, as baseline values were included as covariates.

Table 2 Summary of Adverse Events

Adverse Event	Placebo (n = 40)		PC-SOD (n = 39)	
	No. of Patients	%	No. of Patients	%
General				
Fatigue	30	75	30	77
Malaise	6	15	8	21
Hot flushes	15	38	16	41
Surgery related AEs	7	18	8	21
Change of taste	11	28	8	21
Central nervous system disorders				
Headache	26	65	18	46
Dizziness	8	20	8	21
Gastro-intestinal system disorders				
Nausea	32	80	29	74
Constipation	11	28	11	28
Dyspepsia	7	18	13	33
Mucositis	5	13	9	23
Psychiatric disorders†	10	25	11	28
Respiratory system disorders				
Respiratory tract infections	13	33	17	44
Vision disorders				
(Kerato-) conjunctivitis	18	43	18	46

Note: Adverse events occurring during chemotherapy in more then 20 patients in one of the the 2 treatment groups.
† Nervousness, emotional lability, anxiety, agitation, insomnia, impaired concentration, abnormal thinking, depression and hallucinations

Table 3 Long-term effects

	Overall change over course baseline and follow up		Difference between treatments	95%-confidence interval	p-value
	Placebo	**PC-SOD**			
Echocardiographic parameters					
Left ventricular ejection fraction (%)	-1	-2	-1	-3 to 1	0.31
E/A ratio	-0.0	-0.0	-0.0	-0.1 to 0.0	0.48
Biomarkers of myocardial injury					
NT-proBNP (ng/L, % change)	32.0	14.2	-13.5	-30.9 to 8.2	0.20
CK-MB mass (mg/L, % change)	-5.7	-7.6	-2.0	-11.7 to 8.8	0.70
Electrocardiography					
Heart rate (BPM)	0.6	4.0	3.4	0.4 to 6.5	0.03
QT-interval (msec)	6.8	3.6	-3.1	-11.6 to 5.4	0.46
QTcB-interval (msec, corrected Bazett)	8.8	16.2	7.4	1.9 to 12.9	<0.01
QTcL-interval (msec, corrected Framingham)	7.5	10.8	3.3	-2.1 to 8.7	0.23

*** Significant change from baseline (p < 0.05)**

Table 4 Effects within the courses

	Overall change from course baseline (at 24 hours)		Difference between treatments	95%-confidence interval	p-value
	Placebo	PC-SOD			
Biomarkers of oxidative stress					
Urinary biopyrrin (µmol/g creatinine, % change)	13.0	3.4	10.3	-20.5 to 52.9	0.55
OxLDL (mU/L, % change)	-3.0	3.0	6.2	0.2 to 12.5	0.04
NPBI (µmol/L, % change)	15.9	5.1	-8.5	-22.2 to 7.6	0.28
Biomarkers of myocardial injury					
NT-proBNP (ng/L, % change)	199.8	263.8	21.4	-3.9 to 53.3	0.10
CK-MB mass (mg/L, % change)	8.2	10.3	2.0	-8.1 to 13.1	0.71
Biomarkers of inflammation					
hsCRP (µg/L, % change)	-2.0	-4.8	-2.9	-19.0 to16.5	0.75
sICAM-1 (ng/L, % change)	-1.1	-1.8	-0.7	-2.7 to 1.4	0.50
TNF-a (pg/L, % change)	-23.2	-27.2	-5.3	-18.1 to 9.6	0.46
MIP-1a (pg/mL, % change)	-46.9	-48.6	-3.3	-45.7 to 72.1	0.91
Electrocardiography					
Heart rate (BPM)	-1.2	3.7	4.9	2.2 to 7.6	<0.001
QT-interval (msec)	16.3	5.3	-11.0	-18.2 to -3.9	0.003
QTcB-interval (msec)	14.2	17.1	3.0	-2.5 to 8.4	0.23
QTcL-interval (msec)	14.2	11.5	-2.7	-7.4 to 1.9	0.24

* Significant change from baseline (p < 0.05)

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.

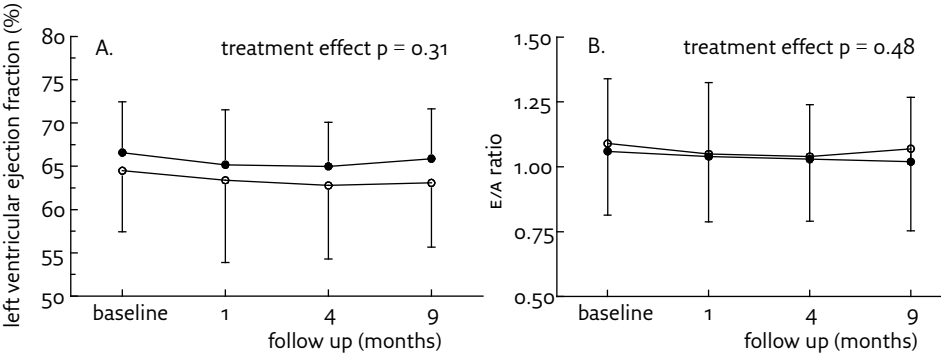

Figure 2 Mean NT-proBNP (a) concentrations, ng/L, and QTc (b), milliseconds, linear corrected for heart rate according to Framingham, during chemotherapy and follow up for PC-SOD (open circles) and placebo (closed circles) and 95%-confidence intervals (PC-SOD up, placebo down) during the course and 1,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