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RESEARCH PAPER

Dexrazoxane prevents doxorubicin-induced long-term cardiotoxicity and protects myocardial mitochondria from genetic and functional lesions in rats

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Background and purpose: Doxorubicin causes a chronic cardiomyopathy in which reactive oxygen species (ROS) accumulate over time and are associated with genetic and functional lesions of mitochondria. Dexrazoxane is a cardioprotective iron chelator that interferes with ROS production. We aim to analyze the effects of dexrazoxane on mitochondria in the prevention of doxorubicin-induced chronic myocardial lesions.
Experimental approach: Wistar rats (11 weeks of age) were injected with intravenous doxorubicin (0.8 mg kg^{-1} weekly for 7 weeks) with or without simultaneous dexrazoxane (8 mg kg^{-1}). Animals were killed at 48 weeks. Cardiomyopathy was scored clinically and histologically and cardiac mitochondria were analyzed.
Key results: Compared to control rats receiving saline, rats treated with doxorubicin alone developed a clinical, macroscopic, histological and ultrastructural cardiomyopathy with low cytochrome c-oxidase (COX) activity (26% of controls). The expression of the mtDNA-encoded COX II subunit was reduced (64% of controls). Myocardia exhibited a high production of ROS (malondialdehyde 338% and superoxide 787% of controls). Mitochondria were depleted of mitochondrial DNA (mtDNA copy number 46% of controls) and contained elevated levels of mtDNA deletions. Dexrazoxane co-administration prevented all these effects of doxorubicin on mitochondria, except that hearts co-exposed to doxorubicin and dexrazoxane had a slightly lower mtDNA content (81% of controls) and mtDNA deletions at low frequency.
Conclusions and Implications: Dexrazoxane prevented doxorubicin induced late-onset cardiomyopathy and also protected the cardiac mitochondria from acquired ultrastructural, genetic and functional damage.
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Keywords: cardiomyopathy; energy metabolism; mitochondria; oxidative phosphorylation; oxygen radicals; cytochrome *c*-oxidase

Abbreviations: COX, cytochrome *c*-oxidase; CS, citrate synthase; GAPDH, glyceraldehyde phosphate dehydrogenase; MDA, malondialdehyde; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; ROS, reactive oxygen species; SDH, succinate dehydrogenase

Introduction

Doxorubicin (adriamycin) is an anthracycline, which is widely used for the treatment of various cancers (Singal and Iliskovic, 1998). Repeated administration of doxorubicin is limited by its dose-dependent, late-onset and irreversible cardiomyopathy. The cardiotoxicity of doxorubicin is thought to result from the generation of reactive oxygen species (ROS), which are catalysed by a complex of doxorubicin with iron. Dexrazoxane (ICRF-187, Zinecard) is an intracellular iron chelator that binds free iron (Fe^{2+} and Fe^{3+}) and removes iron from its complex with doxorubicin, thereby reducing the formation of hydroxyl radicals and superoxide. When co-administered with doxorubicin, dexrazoxane is cardioprotective (Dawson, 1975; Speyer *et al*., 1992; Wexler *et al*., 1996; Swain *et al*., 1997).

We have recently demonstrated that doxorubicin induces heart-specific mutations and quantitative defects in mitochondrial DNA (mtDNA) (Lebrecht *et al*., 2003, 2005). These effects result in a heart-specific impairment of mtDNA-encoded respiratory chain subunits and consequent

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respiratory chain dysfunction. Defects in the respiratory chain promote the liberation of ROS. Oxidative stress is known to induce mtDNA lesions and therefore closes a vicious circle with perpetuated insults to mitochondria (Corral-Debrinski *et al*., 1991). For these reasons and the fact that accumulating mitochondrial lesions are highly correlated with the onset of clinical cardiomyopathy, somatically acquired mtDNA lesions are thought to play an important role in the 'dose memory' of doxorubicin and the clinical onset of the cardiomyopathy (Lebrecht *et al*., 2003).

As pointed out above, dexrazoxane interferes with the formation of ROS at the doxorubicin-iron complex. Acute or subacute effects of dexrazoxane on myocardial mitochondria have been investigated *in vitro* (Hasinoff *et al*., 2003), but the mitochondrial effects of dexrazoxane have not been investigated in truly chronic cardiomyopathy. In the present study, we analysed whether or not dexrazoxane also shielded mitochondria from the effects of chronic doxorubicin exposure and demonstrated the protection of rat organelles from late-onset ultrastructural, genetic and functional damage. These results have important implications for the pathogenetic model of the doxorubicin-induced late-onset cardiomyopathy, the preclinical screening of new doxorubicin analogues and the development of cardioprotective agents aimed at increasing the therapeutic index of anthracyclines.

Methods

Animals

The investigation was approved by the State animal ethics board and conforms to the Guide for the Care and use of laboratory animals published by the US National Institute of Health. Male Wistar rats (Charles River, Sulzfeld, Germany) were fed a normal rat chow (SSniff R/M-H, Spezialdiäten, Germany) and housed in a normal night–day rhythm under standard conditions of temperature and humidity. At 10 weeks of age, all rats received an intravenous cannula (Rat-O-Port, Uno Roestvaststaal, Netherlands) under anaesthesia with Forene (Abbott Park, IL, USA). At 11 weeks of age, the rats were divided into three experimental groups. The animals of group A ($n=9$) served as controls and received seven weekly injections of saline (300–700 μl) through the cannula. Group B rats ($n=15$), received equivalent volumes of doxorubicin (0.8 mg kg^{-1}), freshly reconstituted in water from lyophilized powder (Pharmacia, Germany) as published (Lebrecht *et al*., 2003). Group C rats ($n=10$) received doxorubicin in a protocol identical to group B, but were co-treated with 8 mg kg^{-1} of dexrazoxane (Zinecard, Pharmacia, Kalamazoo, MI, USA). This doxorubicin:dexrazoxane dosage ratio was derived from earlier pharmacokinetic studies (Hochster *et al*., 1992), clinical trials (Speyer *et al*., 1992; Swain *et al*., 1997) and the recommendations on the material data sheet of Zinecard. All rats were killed by cervical dislocation at 48 weeks of age, immediately before post-mortem examination and organ collection. Heart weights were recorded. Left ventricle and apex were snap frozen and cryopreserved in liquid nitrogen until subsequent analysis. Tissue samples were fixed in glutaraldehyde (3%) for subsequent electron microscopy.

Cardiomyopathy score and mitochondrial ultrastructure

Apical heart sections (4 μm) were stained with haematoxylin and eosin and scored for myocardial lesions on a qualitative/quantitative morphological grading scale (Della Torre *et al*., 1996). The scoring was carried out without knowledge of the treatment status of the animals. Within each group, two randomly selected samples of the left anterior ventricle were examined by electron microscopy as described by Lebrecht *et al*. (2003).

Enzyme activities

Cytochrome *c*-oxidase (COX) is a respiratory chain multisubunit enzyme (complex IV), which is encoded partly by nuclear DNA (nDNA) and partly by mtDNA. Succinate dehydrogenase (SDH) is also a respiratory chain enzyme (complex II), but encoded entirely by nDNA. Citrate synthase (CS) is a nDNA-encoded component of the Krebs cycle and located in the mitochondrial matrix. The enzyme activities of COX, SDH and CS were measured spectrophotometrically in freshly prepared tissue extracts, as described by Silvestri *et al*. (1994).

MtDNA-encoded respiratory chain subunits

The expression of the mtDNA encoded subunit I of COX (COX I) was quantified by immunoblot and normalized to the subunit IV of COX (COX IV), which is encoded by nDNA. The blots were also probed with an antibody against glyceraldehyde phosphate dehydrogenase (GAPDH), a cytosolic enzyme encoded by nDNA. Details are described elsewhere (Capaldi *et al*., 1995; Walker *et al*., 2002).

Copy numbers of wild-type and deleted mtDNA

Total DNA was extracted with the QIAamp DNA isolation kit (Qiagen, Hilden, Germany). MtDNA and nDNA copy numbers were determined by quantitative PCR using the ABI 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA). Wild-type mtDNA was amplified between nucleotide positions 2469 and 2542 with the forward primer, 5′-AATGGTTCGTTTGTTCAACGATT-3′ and the backward primer 5′-AGAAACCGACCTGGATTGCTC-3′. MtDNA was quantified with a FAM-fluorophore-labelled probe (5′-6FAM-AAGTCCTACGTGATCTGAGTT-TAMRA-3′). For the detection of nDNA, we selected GAPDH between nucleotide positions 494 and 671, using the forward primer 5′-TGCACCACCAACTGCTTAG-3′ and the backward primer 5′-GGATGCAGGGATGATGTTC-3′. In this case, we used a HEX-fluorophore-labelled probe (5′-HEX-CAGAAGACTGTG GATGGCCCCTC-TAMRA-3′). Each 25 μl reaction contained 20 ng of genomic DNA, 100 nM probe, 200 nM primers and Taq-man Absolute Master Mix (Abgene, Hamburg, Germany). Triplicate amplifications of mitochondrial and nuclear products were performed separately in optical 96-well plates (Applied Biosystems). An initial incubation at 50°C for 2 min was followed by 10 min at 95°C and 40 denaturing steps at 95°C (15 s), alternating with combined annealing/extension at 60°C (1 min). Absolute wild-type mtDNA and nDNA copy numbers were calculated using

serial dilutions of plasmids with known copy numbers (Hammond *et al*., 2003).

The sequence of normal rat mtDNA contains direct repeats between which a 4834-base pair (bp) deletion may be deleted by slipped mispairing during replication (Schon *et al*., 1989). This deletion is similar to the age-related 'common' 4977-bp deletion in humans. The common mtDNA-deletion in rats was probed by PCR using extra-deletional primers and short extension cycles as described earlier (Lebrecht *et al*., 2003). The identity of the PCR product was confirmed by sequencing (MWG Biotech, Germany).

ROS production

Malondialdehyde (MDA) is one of the end products of lipid peroxidation and an indicator of ROS production. MDA was quantified in tissues with a spectrophotometric assay for thiobarbituric acid-reactive material (Tuzgen *et al*., 1998).

Superoxide was measured on transverse sections of the left ventricle with the fluorescent dye dihydroethidium at an excitation wavelength of 510 nm (Sigma, Taufkirchen, Germany) (Miller *et al*., 1998). The intensity of the fluorescence was quantified using Scion Image (Scion Corp., Frederick, MD, USA).

Statistics

Group means were compared by unpaired *t*-test or Wilcoxon analysis; nonlinear exponential regression analysis was computed using the Sigma Plot 2000, version 6.0 (SPSS Inc., Chicago, IL, USA) statistical package. Values of $P<0.05$ were taken as showing significant differences between means.

Results

Rat survival and post-mortem morphology

Five of the fifteen rats from the doxorubicin group B died between weeks 32 and 46, before the predetermined end point at week 48. Post-mortem examination revealed pleural effusions and myocardial dilatation in three of these rats. One of the 10 animals in the group C died in week 38, showing a similar macroscopic pathology. All these rats were excluded from the analysis because we were unable to control for post-mortem time.

Unlike the controls, showing no clinical signs of myocardial failure at week 48, five of the 10 group B rats available for analysis had an increased respiratory rate, nine had macroscopic evidence of myocardial dilatation and eight had pleural effusions. The livers of all 10 group B animals were enlarged and of a dark red, engorged appearance. None of the dexrazoxane co-treated rats had respiratory or hepatic pathology.

The mean heart weight among the doxorubicin-exposed animals was significantly elevated in those treated without dexrazoxane, but not in those treated with dexrazoxane (Table 1). Similarly, the degree of myocardial damage, as assessed with the cardiomyopathy score, was substantially elevated in group B, but not in rats treated with dexrazoxane (Table 1). Electron microscopy of hearts exposed to doxorubicin alone revealed a disorganized myofibrillar lattice with interspersed clusters of mitochondria. The organelles frequently contained no cristae but large deposits of electron-dense material (Figure 1). Animals that were co-administered dexrazoxane had only slightly increased numbers of mitochondria, but none of the other ultrastructural pathology.

Respiratory chain activity

In group B, the mean COX activity was 26% of control values, whereas there was no decrease of COX activity within animals co-treated with dexrazoxane (Table 2). The mean SDH activity was unchanged in both groups B and C myocardia. When the enzymatic activity of COX was normalized to the enzymatic activity of SDH, the mean COX/SDH ratio was severely reduced in group B, but not group C hearts, compared with controls. The enzymatic activity of CS was slightly increased in group B myocardia (126% of control values), but not in group C.

Among all rats, the histological degree of myocardial damage correlated inversely with both, the absolute COX activity ($r=-0.71$, $P<0.001$) and the COX/SDH ratio (Figure 3a).

Table 1 Protective effects of dexrazoxane on heart mitochondria

	Control	*Doxorubicin (B)*	*Doxorubicin + dexrazoxane (C)*	P-*value (B vs control)*	P-*value (C vs control)*	P-*value (C vs B)*
Heart weight (g)	1.25±0.11	1.83±0.12	1.41±0.15	0.004	0.12	<0.001
Cardiomyopathy score	1.6±1.1	7.2±2.5	1.5±1.0	<0.001	0.91	<0.001
COX I/COX IV[a]	100±15	64±22	93±13	<0.001	0.27	0.003
COX IV/GAPDH[a]	100±15	100±14	95±20	0.99	0.56	0.53
mtDNA copies/cardiomyocyte	676±117	314±119	549±120	<0.001	0.04	<0.001
'Common' mtDNA-deletion	−	++	(+)	NA	NA	NA
MDA[b]	45.5±17.2	153.6±59.6	47.3±22.6	<0.001	0.86	<0.001
Superoxide[a]	100±33	787±282	164±101	<0.001	0.09	<0.001

Abbreviations: COX, cytochrome *c*-oxidase; GAPDH, glyceraldehyde phosphate dehydrogenase; MDA, malondialdehyde; mtDNA, mitochondrial DNA.
The values indicate group means±s.d. The 'common' mtDNA-deletion was detected in two animals of group C (+) and at higher levels in all rats of group B (++), but not in control animals.
[a]% of control mean;
[b]μmol g tissue^{-1}.

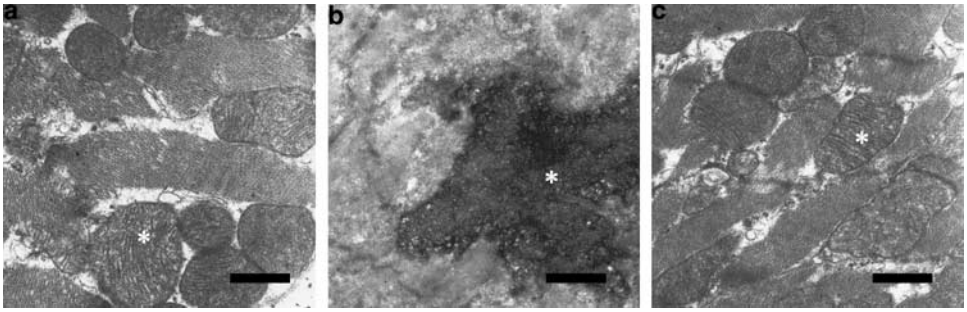

Figure 1 Representative electron micrographs of hearts from untreated control animals (**a**), the doxorubicin only group (**b**) and the doxorubicin plus dexrazoxane group (**c**). Mitochondria are marked with a star. Scale bar: 0.6 μm.

Table 2 Activity of mitochondrial enzymes in heart

	Control	*Doxorubicin (B)*	*Doxorubicin + dexrazoxane (C)*	P-*value (B vs control)*	P-*value (C vs control)*	P-*value (C vs B)*
COX[a]	54 ± 24	14 ± 10	45 ± 12	< 0.001	0.30	< 0.001
SDH[a]	60 ± 26	75 ± 39	51 ± 17	0.37	0.38	0.11
COX/SDH-ratio[b]	100 ± 18	26 ± 22	99 ± 16	< 0.001	0.88	< 0.001
CS[a]	3440 ± 470	4346 ± 694	3730 ± 433	0.004	0.19	0.04

Abbreviations: COX, cytochrome *c*-oxidase; SDH, succinate dehydrogenase.
The values represent group means ± s.d.
[a] μmoles min^{-1} g^{-1} protein^{-1};
[b]% of control mean.

MtDNA-encoded respiratory chain subunits

The mean COX I/COX IV-ratio was reduced in group B; this change was prevented by dexrazoxane co-treatment (Table 1). The COX IV/GAPDH ratio did not statistically differ between all groups. The COX I/COX IV ratio was positively correlated with both, the absolute COX activity ($r = 0.49$, $P = 0.03$) and the COX/SDH ratio ($r = 0.49$, $P = 0.03$) and inversely correlated with the cardiomyopathy score (Figure 3b). These results indicate that the respiratory chain defect mediated by doxorubicin is restricted to the mtDNA-encoded respiratory chain subunits and prevented by dexrazoxane.

Wild-type and deleted mtDNA

Wild-type mtDNA copy numbers in hearts exposed to doxorubicin were approximately half of those in controls (Table 1). Dexrazoxane co-injection significantly prevented mtDNA loss, but did not fully prevent mtDNA depletion (residual mtDNA amounts being 81% of control values). Among all rats, the myocardial mtDNA copy numbers correlated inversely with the cardiomyopathy score (Figure 3c), and positively with the absolute COX activity ($r = 0.57$, $P = 0.002$), the COX/SDH ratio ($r = 0.62$, $P = 0.02$) and the COX I/COX IV ratio ($r = -0.74$, $P < 0.001$).

A 459 bp PCR product was identified in the hearts of all animals treated with doxorubicin alone and in two myocardia of rats co-treated with dexrazoxane (Figure 2). Whereas the intensity of the PCR product was high in the former group, it was low in the latter. Sequencing confirmed that the 459 bp PCR products comprised a mtDNA fragment, in which 4834 bp had been deleted (for example the 'common' deletion). No deletions were detected in any of the control hearts.

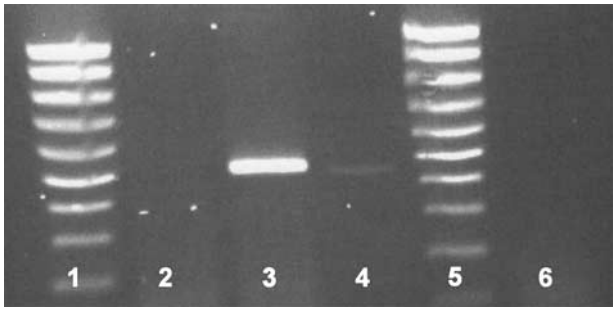

Figure 2 Analysis of representative PCR products by agarose gel electrophoresis, as a correlate of the 'common' mtDNA-deletion. DNA standard (100 bp) (Sigma, Taufkirchen, Germany) was added to lanes 1 and 5. PCR products from control (lane 2) and group B myocardia (lane 3). PCR products from one of the two group C hearts, in which the 'common' deletion was detectable (lane 4). No template (lane 6).

Reactive oxygen species

In the absence of dexrazoxane, doxorubicin-exposed myocardia exhibited increased levels of MDA as an indirect indicator of ROS formation (by a factor of 3.4. compared with controls). This effect of doxorubicin was prevented by co-administration of dexrazoxane (Table 1). Myocardial MDA levels were inversely correlated with the COX enzyme activity ($r = -0.46$, $P = 0.01$), the COX/SDH ratio ($r = -0.71$, $P < 0.001$), the COX I/COX IV ratio ($r = -0.71$, $P < 0.001$) and the mtDNA content ($r = -0.77$, $P < 0.001$), and positively correlated with the cardiomyopathy score (Figure 3d).

Myocardial superoxide correlated with the MDA levels (Figure 3e) and was increased by 787% in group B compared with controls (Table 1). Although superoxide levels were also slightly elevated in hearts exposed to doxorubicin plus

dexrazoxane (164% of the control mean), this increase did not reach statistical significance ($P=0.09$). Among all animals, myocardial superoxide levels correlated positively with the cardiomyopathy score (Figure 3f) and negatively with the COX activity ($r=-0.71$, $P<0.001$), the COX/SDH ratio ($r=-0.87$, $P<0.001$), the COX I/COX IV ratio ($r=-0.64$, $P<0.001$) and the mtDNA/nDNA ratio (−0.69, $P<0.001$).

Discussion

We have previously investigated the pathogenesis of the chronic doxorubicin cardiomyopathy in a rat model and demonstrated that the clinical onset of myocardial failure is associated with increased ROS production and mitochondrial lesions that continue to accumulate with time even in the absence of continued anthracycline applications (Lebrecht *et al*., 2003). Whereas previous investigators mostly examined dexrazoxane on the acute or subacute cardiac effects of doxorubicin, we have now examined the effects of dexrazoxane on the somatically acquired mitochondrial lesions and ROS production in chronic cardiotoxicity and demonstrate that the membrane permeable iron chelator protected against almost all mitochondrial injury.

From the literature, it is clear that acute doxorubicin exposure induces lesions on the mitochondria of cardiomyocytes and that this damage can be prevented by

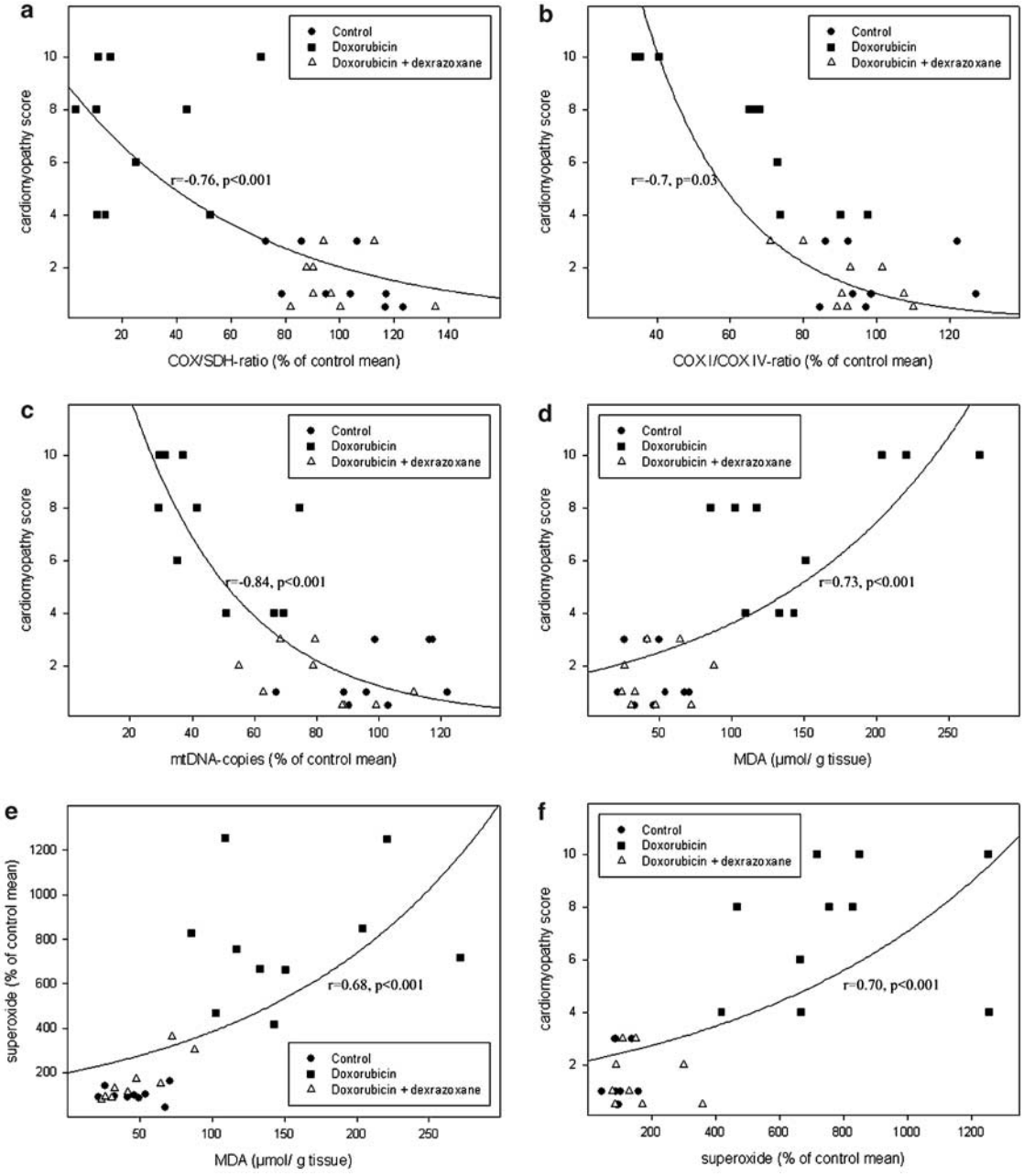

Figure 3 Correlations among cardiac damage, parameters of mitochondrial integrity, superoxide levels and lipid peroxidation in all rats.

dexrazoxane. *In vitro* work on isolated rat cardiomyocytes has shown that doxorubicin leads to a rapid depolarization of the mitochondrial membrane potential and that this effect can be prevented by dexrazoxane (Hasinoff, 1990). Similarly, using submitochondrial particles of bovine hearts, it was recently shown that the activity of nicotinamide adenine dinucleotide phosphate (NADH) cytochrome *c* reductase, a respiratory chain enzyme encoded by both mtDNA and nDNA, was impaired within minutes after incubation with a Fe^{3+}-doxorubicin complex. This effect on NADH-cytochrome *c* reductase was also preventable by chelating iron (Hasinoff, 1990). The rapid kinetics of mitochondrial impairment after acute doxorubicin exposure however cannot be explained by effects on mtDNA and are thus more likely to result from either direct ROS-mediated respiratory complex inactivation or from the ability of the Fe^{3+}-doxorubicin complex to bind to cardiolipin, a lipid that is most abundant in the inner mitochondrial membrane of heart and important to respiratory function (Goormaghtigh *et al*., 1990; Keizer *et al*., 1990).

We have observed chronic effects of doxorubicin on mtDNA. Oxidative stress can interfere with the normal function of gamma-polymerase, the enzyme that replicates mtDNA (Graziewicz *et al*., 2002). Such prolonged mtDNA replication is thought to promote slip replication and to foster the acquisition of mtDNA deletions (Schon *et al*., 1989). Therefore, increased oxidative stress is an explanation for the presence of the observed mtDNA mutations. The intramyocardial half-life of doxorubicin and its metabolites is however only a few hours (Johnson *et al*., 1986) and suggests a different mechanism of ROS formation in the late-onset cardiomyopathy. Any respiratory chain damage promotes the generation of ROS. ROS-mediated mtDNA insults then lead to a further decline in respiratory function. This vicious circle can be initiated during acute doxorubicin exposure and then be perpetuated by oxidative stress, even in the absence of doxorubicin. This mechanism also explains the delayed onset of the cardiomyopathy in patients with inherited mtDNA mutations (Larsson *et al*., 1990).

Peroxynitrite ($ONOO^-$) is a highly reactive oxidant, which is formed from nitric oxide and superoxide (Ferdinandy, 2006). Peroxynitrite has many effects but also induces lipid peroxidation, promotes DNA strand breakage, inhibits the respiratory chain and causes apoptosis (Pacher *et al*., 2005; Ferdinandy, 2006). The importance of peroxynitrite for the development of doxorubicin cardiomyopathy is underlined by the observation that, in acute models of cardiotoxicity, interventions that decrease peroxynitrite are cardioprotective (Pacher *et al*., 2003; Andreadou *et al*., 2007). It is therefore conceivable that the dexrazoxane-mediated block of superoxide formation interferes with the formation of peroxynitrite.

Interestingly, dexrazoxane also inhibits topoisomerase II, an enzyme that is necessary to unwind the normal entwinement of nDNA duplexes during chromosomal replication (van Hille *et al*., 2000; Classen *et al*., 2003; Jensen *et al*., 2004). Mitochondria possess a similar topoisomerase and an impairment of the mitochondrial topoisomerase (also called 'twinkle') was shown to cause reductions in mtDNA copy number and deletions within mtDNA (Tyynismaa *et al*., 2004, 2005). Although not previously investigated, it is likely that dexrazoxane as a membrane permeable neutral molecule also enters mitochondria (Dawson, 1975), where it may also inhibit twinkle. Thus, dexrazoxane-mediated inhibition of twinkle may have contributed to the low-frequency mtDNA deletions in the group C rats. As mtDNA deletions are thought to persist and even accumulate, over a lifetime in heart and other post-mitotic tissues (Dawson, 1975), an effect of dexrazoxane on mtDNA may be observed after many months, despite the fact that the half-life of dexrazoxane predicts its almost complete elimination after a few hours (Hochster *et al*., 1992; Schroeder and Hasinoff, 2002). It is possible however that dexrazoxane does not completely block all the effects of doxorubicin, which is also known to inhibit the topoisomerase type II (Tewey *et al*., 1984; Keizer *et al*., 1990) and to form mtDNA adducts and crosslinks (Cullinane *et al*., 2000).

In our analysis, we focused on mtDNA and its respiratory chain gene products, although other mechanisms are thought to contribute to the delayed onset of chronic doxorubicin cardiomyopathy. For example, proapoptotic factors of mitochondrial origin have recently been associated with superoxide production and were implicated in the execution of cardiomyocyte death (Childs *et al*., 2002; Yi *et al*., 2006). As the proapoptotic effects of doxorubicin may also result from mitochondrial failure, such additional effects do not contradict, but may rather complement our findings.

Our study results should also not be generalized, because gender- and age-specific differences in susceptibility to doxorubicin were demonstrated in a subacute model of cardiotoxicity, with female and younger rats being more sensitive (Heon *et al*., 2003). Furthermore, the optimal dose of dexrazoxane has not been determined. Other investigators have however found that dexrazoxane is well tolerated and that a 20:1 dexrazoxane–doxorubicin ratio may offer an even better subacute cardioprotection (de Beer *et al*., 2002). No side effects were observed with dexrazoxane doses exceeding ours by a factor of five ($40\,mg\,kg^{-1}\,week^{-1}$) (de Beer *et al*., 2002). All adverse effects in our rats treated with dexrazoxane plus doxorubicin were attributable to doxorubicin alone. These findings, the good tolerability of dexrazoxane in clinical trials (Speyer *et al*., 1992) and the clinically proven efficacy in preventing doxorubicin-induced late-onset cardiotoxicity, strongly support the use of dexrazoxane in patients who receive doxorubicin in a cardiotoxic cumulative dose and who are estimated to survive beyond chemotherapy.

In conclusion, the results of our study suggest that dexrazoxane prevents from late-onset doxorubicin cardiomyopathy by protecting cardiac mitochondria from interconnected genetic and functional insults, which are initiated and perpetuated by ROS.

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Conflict of interest

The authors state no conflict of interest.

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